Chapter 2

Biological Control of Parasites

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Abstract

Parasites (ectoparasites or endoparasites) are a major cause of diseases in man, his livestock and crops, leading to poor yield and great economic loss. To overcome some of the major limitations of chemical control methods such as rising resistance, environmental and health risks, and the adverse effect on non-target organisms, biological control (biocontrol) is now at the forefront of parasite (pests) control. Biocontrol is now a core component of the integrated pest management. Biocontrol is defined as "the study and uses of parasites, predators and pathogens for the regulation of host (pest) densities". Considerable successes have been achieved in the implementation of biocontrol strategies in the past. This chapter presents a review of the history of biocontrol, its advantages and disadvantages; the different types of biological control agents (BCAs) including predators, parasites (parasitoids) and pathogens (fungi, bacteria, viruses and virus-like particles, protozoa and nematodes); the effect of biocontrol on native biodiversity; a few case studies of the successful implementation of biocontrol methods and the challenges encountered with the implementation of biocontrol and future perspectives.

Keywords: biological control, biological control agents, parasites, humans, plants, livestock, case study, challenges

1. Introduction

In nature, the population size of every species is regulated by natural environmental factors. These factors are responsible for the "checks and balances" of a population of living organisms. The event where living organisms live and die a natural death unaided by man is termed "natural control". Weather (abiotic or non-living factors) is an important factor in natural control; temperature and humidity are determinants of the survival of living organisms. Availability of competition (biotic factors) is also an important determinant for the survival of living organisms [1]. Many organisms are killed by pathogens (disease-causing agents) such as bacteria, viruses, fungi, parasites (parasitoids) and predators [1].

Living organisms, which are considered undesirable, are generally referred to as pests. Environmental factors (such as weather, geography and soil conditions) which affect pest populations generally vary from one location to another and changes through time. A combination of these factors may substantially reduce the pest population in one geographical area and make it more abundant in another. Pests sometimes outwit their natural enemies and grow to very high population density. To keep their population in check will necessitate the manipulation of the population of their natural enemies by man. This is termed biological control or simply biocontrol. Biocontrol is therefore defined as "any activity of one species that reduces the adverse effect of another" [1]. Biocontrol can also be defined as "the study and uses of parasites, predators and pathogens for the regulation of host (pest) densities" [2]. Biological control differs from natural control in that the latter does not involve human manipulation. The organism that suppresses the pest population is generally referred to as a biological control agent (BCA).

A parasite is an organism that lives and feeds in or on a host [3]. Parasites that invade and live within the host are referred to as endoparasites; meanwhile, those that live on the surface without invading the host are referred to as ectoparasites. Endoparasites include helminths and protozoa, and ectoparasites are fleas, ticks, mites, insects and so on. Parasites are a major cause of disease in man, his livestock and crops, leading to poor yield and economic loss. The biocontrol of parasites therefore entails the use of BCAs to suppress the population of the parasites.

This chapter focuses on the biological control of parasites, providing a brief history of biocontrol; their advantages and disadvantages; types of BCAs including predators, parasites (parasitoids) and pathogens (fungi, bacteria, viruses and virus-like particles, protozoa and nematodes); their effect on the native biodiversity; a few case studies of successful implementation of biocontrol; challenges encountered with the implementation of biocontrol strategies and finally their future perspectives.

2. History of biological control

The concept of biological control is not entirely new. The ancient Egyptians were probably the first to employ biocontrol dating some 4000 years ago, when they observed that cats fed on rodents, which damaged their crops. This most likely led to the domestication of the house cat [4]. However, the first record of biocontrol is from China. As early as the third century, a nest of the ants *Oecophylla smaragdina* were sold near Canton (today known as Guangzhou in China) for use in control of the citrus insect pests such as *Tesseratoma papillosa* (Lepidoptera). By 1200 A.D., the usefulness of the ladybird beetles as biological control agents of aphids and scales had been recognized. Between 1300 and 1799 A.D., the importance of biological control tools was recognized. Van Leeuwenhoek was probably the first to describe insect parasitism, which he illustrated in his publication in 1701. In 1726, de Reaumur recognized the first insect pathogen, a Cordyceps fungus that infects noctuid. The mynah bird, *Acridotheres tristis*, was successfully introduced from India to Mauritius by the British and the French for the

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control of the red locust, *Nomadacris septemfasciata*, in 1762 (see **Figure 1**). In 1776, in Europe, the control of the bedbug, *Cimex lectularius*, was successfully accomplished by the release of the predatory pentatomid, *Picromerus bidens*. Koller was the first to put forth the concept of "natural control" in 1837 [5].

Between 1850 and 1887, the concept of biological control switched to the United States. In 1870, Charles V. Riley was the first person to conduct the successful movement of parasitoids for biological control when parasitoids were moved from Kirkwood, Missouri, to other parts of the United States for the control of the weevil (*Conotrachelus menuphar*). In 1883, the US Department of Agriculture (USDA) imported *Apanteles glomeratus* from England to control *Pieris rapea* (the imported cabbageworm) parasites that were distributed in DC, Iowa, Nebraska and Missouri. This marked the first intercontinental shipment of parasites. It was not until the 1800s that well-thought-out biological control projects were implemented across Europe. In 1888, the cottony cushion scale project was launched to control the cottony cushion scale, *Icerya purchase*, which was threatening to destroy the infant citrus industry. This was the first project to be launched. Since then, many projects have been launched including the gypsy moth project in New England (1905–1911) [5].

Figure 1. Images of some common parasites/pests (centre cycle) and biological control agents (external cycles) used in the biocontrol of parasites.

Between 1930 and 1940, there was a peak in biological control activity in the world with 57 different biological control agents established at various places. During World War II, there was a sharp drop in biological control activity and after the war, biological control did not regain popularity due to the production of relatively inexpensive synthetic pesticides. It was not until the late 1960s that the concept of integrated pest management (IPM) was implemented, and biological control was seen as a core component of IPM by some [5]. The other components of IPM are habitat manipulation, modification of cultural practices and the use of resistant varieties.

3. Importance of the biological control of parasites

Control of parasites nowadays is mainly by the use of chemicals (pesticides), but the commonly used chemicals are fast losing their effectiveness as a result of resistance arising from indiscriminate use. Moreover, pesticides present a danger to people, the environment, their residual build-up and their effect on non-target organisms such as beneficial insects, birds, domestic animals and sometimes the crop itself. A suitable alternative to the growing problem is biological (natural) control. Under ideal conditions, biocontrol has sustainability, which is lacking in the other methods of parasite control. There are several methods through which biological control of parasites could be achieved, including the use of predators (such as arthropods, mites, flies, beetles, amphibians, fish, birds, rodents, etc.), parasites (parasitoids) and pathogens (such as fungi, bacteria, viruses and virus-like particles, protozoa and nematodes).

4. Advantages and disadvantages of biocontrol

4.1. Advantages of biocontrol

Biocontrol offers some advantages over other pest-control strategies, particularly chemical pesticides. These advantages include as follows:

(1) It is environmentally friendly and safe to the applicator.

(2) There are no residues.

(3) Biocontrol could be very economical in some cases.

(4) Biocontrol is easy to apply; in many cases, we are merely manipulating something to favour naturally occurring controls.

(5) Biological control is sometimes lasting, thereby eliminating the needs for continuous re-application as is necessary with pesticides.

(6) Biocontrol is easily established

(7) BCAs are frequently very host specific.

(8) Unlike chemical methods, pests do not become resistant against BCAs.

4.2. Disadvantages of biocontrol

The disadvantages of biocontrol include as follows:

(1) Biocontrol is often slow. In biocontrol of pests, there is often a lag time between build-up of the pest population and build-up of the biocontrol agent. If a pest population is already at or above economically damaging levels, pesticides are the only alternative.

(2) BCAs do not completely eliminate their host [6]. If they do, they would also die. However, biological control may be integrated with other pest control strategies to achieve complete eradication.

(3) With biocontrol, there is the possibility that the BCA may tend to feed on the desired plants or insect, that is, crossovers [7–9]. Careful selection of the BCA will minimize this problem.

(4) BCAs are frequently ineffective in multiple weed complexes when used in biocontrol of weeds. This may be because the weed and the crop are so closely related that the control agent affects both the pest and the crop.

(5) The shipping, storage and application techniques of BCA can be relatively complex. Production of the BCA is also costly in some cases.

(6) Biocontrol sometimes may be costly compared to conventional methods. The high cost is usually attributed to the research that has to be done prior to implementation of the biocontrol strategy.

(7) Biocontrol if not well conceived may lead to dramatic changes in native biodiversity.

5. Types of biological control agents

Biocontrol of insects may include predators (e.g. spiders), parasites (parasitoids) or pathogens like viruses, bacteria, fungi, protozoa and nematodes (see **Figure 1**).

5.1. Predators

Predators can be vertebrates or invertebrates, some of which are arachnids, but deployment of insects is most common. The efficiency of predators in controlling populations of some ticks in different habitats varies and may reach up to 100% [10, 11]. For example, predation has been observed to be lower in tall grass areas than in short grass areas [12]. Likewise, predation has been observed to be two to eight times higher in open areas than in thick pasture areas and non-intensive pasture or agricultural areas [13]. The different types of predators can be classified as invertebrates and vertebrates.

5.1.1. Invertebrates

5.1.1.1. Spiders and insect herbivores

Spiders prey on many insects. Spiders have a defined habitat; a change in the habitat such as mulching may increase their population by as much as 60% [14, 15]. River prawns have been observed to prey on snails [16]. Insect herbivores including the cell-content feeder *Liothrips ludwigi* (Thysanoptera), the stem borers *Merocnemus binotatus* (Boheman) and *Tyloderma* spp. (Coleoptera) have shown promise in the control of weeds [17]. Both the adult and larval stages of the predatory thrips, *Scolothrips sexmaculatus,* are known to feed on spider mites and other thrips [18]. *S. sexmaculatus* prefers spider mite eggs but adult females will consume other mite stages as well [18].

5.1.1.2. Mites

Some mites are nematode predators. For example, some mites (*Phytoseiid* spp.) are capable of consuming *Ascaris* ova in the soil [19]. There are also a few mite species that are voracious predators of eggs and larvae of houseflies and other filth flies that develop in manure and faeces of livestock; for example, *Macrocheles muscaedomesticae* can eat up to 10 housefly eggs per day [20].

5.1.1.3. Flies

Use of the predatory fly, *Hydrotaea (Ophyra) aenescens*, which is commercially available in several northern European countries, presents a breakthrough in the indoor control of the housefly, *Musca domestica* [21]. Small flies such as *Mutilla glossinae* are important parasites of tsetse and are promising BCAs against the tsetse fly [22].

5.1.1.4. Ants

Around 27 species of ants from 16 genera mainly *Aphaenogaster*, *Iridomyrmex*, *Monomorium*, *Pheidol* and *Solenopsis* are known to prey on ticks, horn flies and different agricultural pests [23]. Application of the fire ant, *Solenopsis inucta,* in Louisiana in the USA markedly reduced the population of ticks (*Ixodes* spp.) transmitting anaplasmosis in cattle [23]. However, a wide applicability of fire ants may pose a challenge because of their painful sting.

5.1.1.5. Beetles

Dung beetles of the family Scarabaeidae (Scarabaeinae, Geotropinae and Aphodiinae) are useful in the control of pasture livestock flies since they breed primarily in cow pats. In addition, dung beetles such as *Onthophagus ganelle* and *Euniticellus intermedius*, introduced from Africa to Australia, are regarded as useful in the biocontrol of *Musca vetustissima* and the buffalo fly *Haematobia exigua*. The African dung beetles are well adapted to cattle faeces and compete with fly larvae for food. Furthermore, the rapid burial of dung by the beetles reduces the breeding habitats for the flies [21]. The scarab beetle (*Scarabaeus sacer*), also referred to as sacred scarab among ancient Egyptians, was famous for its habit of rolling balls of dung along the ground depositing them in its burrows. The female would lay her eggs in the ball of dung. When they hatched, the larvae would use the ball for food. When the dung was consumed, the young beetles would emerge from the hole [24]. Dung beetles have been reported to reduce horn flies by as much as 95%, bush flies by 80–100% and result in nine times of fewer parasites produced [25, 26]. Dung beetles can also play a role in the biocontrol of bovine gastrointestinal nematodes (Trichostrongylidae). The spotted lady beetle (*Coleomegilla maculata*) is also able to feed on the eggs and larvae of the Colorado potato beetle *Leptinotarsa decemlineata* [27] and can be used in its control. The larvae of Coccinellids (ladybird or ladybug) are voracious predators of aphids and also consume mites, scale insects and small caterpillars.

5.1.1.6. Dragonflies and water bugs

Dragonflies (see **Figure 1**) may look like scary biters, but they are only dangerous to mosquitoes. Dragonfly larvae, "nymphs", feed on mosquito larvae, and adult dragonflies feed on adult mosquitoes [28]. On the other hand, water bugs, *Diplonychus indicus,* are also known to prey on mosquito larvae [29].

5.1.2. Vertebrates

5.1.2.1. Amphibians and fishes

The water tortoise *Pelomedusa subrufa* has been reported to be able to remove ticks from black rhinos in a streambed [30]. Also in some areas, the mosquito larvae may be controlled biologically by predatory fish such as *Gambusia affinis* and *Guppy poecilia* [31]. One study showed that introducing *Gambusia affinis* into water wells resulted in 98% reduction in the larval density of *Anopheles stephensi* [32]. Other predatory fishes such as the *Cyprinus carpio*, *Ctenopharyngodon idella*, *Aphanius dispar*, *Aplocheilus blocki*, *Tilapia* spp., *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* have also shown promise in the control of mosquitoes [33]. In China, for example, the presence of carp fish in certain rice fields reduced the number of malaria cases [34]. Another predatory fish, the black carp (*Mylopharyngodon piceus*), has shown promise in the biocontrol of the intermediate host snails of fish-borne zoonotic trematodes [35]. Snails (some of which are intermediate hosts of fascioliasis, paramphistomes and schistosomes) are eaten by some fishes as well.

5.1.2.2. Reptilians

Some lizards can eat arthropods. The lizard stomach may contain as many as 2.5–15 ticks/stomach. However, because there are few lizards near the bird nest, their effect on the tick population may be limited [21]. The Australian gecko *Gehydra dubia* and the exotic Asian house gecko *Hemidactylus frenatus* have been observed to prey on mosquitoes; in the laboratory, they have been observed to prey more on female mosquitoes and are therefore a promising tool for the biological control of malaria [28].

5.1.2.3. Birds

Birds are generally thought to be the main predators of insects. Some bird species are known to pick off ticks from the host during flight or collect them from the ground. Birds also eat the larvae of dung flies. One approach for biocontrol of trematodes is the control of the snail intermediate host. Domestic fowls and birds are predators of snails. Scrub jays have been observed to spend 89% of their time searching deer for ectoparasites [36]. In Africa, chickens are natural predators of ticks and actually pick ticks from the bodies of cattle as they lie down as well as from the vegetation [37].

5.1.2.4. Rodents and mammals

Some mammals are insectivorous. As an example, *Sorex araneus* preys on ticks and at times prefers them to alternative foods [12, 38]. Shrews seem to locate hidden ticks by their smell. Mice and rats are often cited as preying on ticks [39]. However, it is worth to mention here that it is not advisable to use rodents for controlling insects as they are more harmful and transmit more diseases than insects.

5.2. Parasites (parasitoids)

Parasites that attack other parasites are generally referred to as parasitoids. Parasitoids are very diverse in appearance, biology and the hosts they attack. Parasitoids lay their eggs on or in the body of an insect host, which is then used as food for the developing larvae. The host is ultimately killed. Most insect parasitoids are wasps or flies and may have a very narrow host range. The most important groups are the ichneumonid wasps, which prey mainly on caterpillars of butterflies and moths; braconid wasps, which attack caterpillars and a wide range of other insects including greenfly; chalcid wasps, which parasitize eggs and larvae of greenfly, whitefly [40], cabbage caterpillars and scale insects and tachinid flies, which parasitize a wide range of insects including caterpillars, adult and larval beetles and true bugs [37, 41–44].

5.3. Pathogens

5.3.1. Fungi

Pathogenic fungi can be classified into two: entomopathogenic fungi and nematopathogenic fungi.

5.3.1.1. Entomopathogenic fungi

Fungi that infect and kill arthropod (insects, ticks or mites) pests are referred to as "entomopathogenic fungi". Over 750 species of entomopathogenic fungi have been identified, a majority of them belong to the phylum Ascomycota and a few to the phylum Zygomycota and Ascomycotina [45]. Unlike the other BCAs, some fungi do not need to be ingested by the host [33]; entomopathogenic fungi produce spores as the insect comes in contact with these spores either on the body of dead insects or surfaces or in the air as airborne particles; the spores germinate in the presence of high humidity and produce germ tubes that allow them to penetrate the cuticle of the insect, usually at joints or creases where the insect's protective covering is thinner [46]. Death usually follows between 4 and 10 days, depending on the type of fungus and the number of infecting spores. Other fungi cause death by the production of toxins (mycotoxin). After death, the fungus produces thousands of new spores on the dead body, which disperse and continue their life cycle on new hosts. Some species go into a resting stage, which survive periods of adverse conditions before forming or releasing spores. The ascomycetes together with the mitosporic fungi are most widely used for biocontrol of pests.

The most commonly investigated entomopathogenic fungi belong to the genera *Metarhizium* and *Beauveria* and are increasingly being used in commercial formulation against arthropods. The Hyphomycetes, *Beauveria bassiana* and *Metarhizium anisopliae* (formerly known as *Enthomphthora anisopliae*) are the most common species known to cause natural outbreaks to a wide range of insect hosts, on their own under favourable conditions. These fungi provide a long-term strategy for larvae and puparia control since they may survive in the soil through recycling in insects or roots [47, 48]. *Metarhizium anisopliae* and *Beauveria bassiana* are also effective in the control of mosquitoes [49]. They infect mosquitoes early in life and kill them, depending on the exposure dose and fungus isolate after 3–14 days [50]. Fungal spores can be applied in outdoor attracting odour traps, on indoor house surfaces and on cotton pieces hanging from ceilings, bed nets and curtains [51, 52] to control adult mosquitoes. Commercially available products based on *B. bassiana* are Mycotrol O (Emerald BioAgriculture), Naturalis Home and Garden (H&G), Naturalis L (Troy BioSciences, Inc.) and Biosect® (Kafr El Zayat—KZ Chemicals, Egypt) [44]. For example, Khater [53] used Biosect® to control larvae of both *Musca domestica* and *Culex pepiens* in-vitro and observed that the total larval mortalities of mosquitoes were almost 100%.

Hyphomycetes of the genera *Fusarium* also contain some important pathogens. For example, Ghannam et al. [54] observed that certain species of *Fusarium* (*F. solani, F. oxysporum and F. arthrosporioides* strain E4a) were able to increase the dead spikes of the obligate holoparasitic weed, broomrape (*Orobanche* spp.), by 33.6–72.7%, thereby making it promising for broomrape control.

Other fungi species that are increasingly being used as BCAs include the Oomycetes, *Lagenidium giganteum* (formerly: *L. culicidum*), which are known to be pathogenic to the larvae of several mosquito genera [55]. Unfortunately, the fungus is not effective for mosquitoes in brackish or organically rich aquatic habitats. In contrast, *Lagenidium* spp. was isolated from Egypt for the first time from *Culex pipiens* larvae infesting a polluted creak in Miet El-Attar, Benha, Egypt, and it was observed to effectively control *Culex pipiens* [53]. As the fungi has the ability of self-propagation, it could be used for effective control of the vector of bancroftian filariasis and rift valley fever virus [46].

The entomophthorales are another group of fungi that are able to cause natural outbreaks in insect populations and are also promising as good BCAs [56]. Several different *Entomophthora muscae* sensu stricto genotypes have been documented, and each type has demonstrated a high degree of host specificity [56]. All available literature deal with *E. muscae* as a pathogenic fungi of adult *Musca domestica*, but Khater [53] isolated, for the first time in Egypt, from

Moshtohor, Toukh and Qlubia governorate a strain that has the unique ability to infect larvae of the house flies. Fungi such as *Leptolegnia* spp., *Coelomomyces* spp., *Hirsutella thomsonii*, *Nomuraea rileyi* and *Vericillum lecanii* are also being used in the control of insects [46].

The ascomycetes, *Trichoderma harzianum* and *T. viride,* have been shown to antagonize the fungi causing damping-off and wilt in bean plants [57].

5.3.1.2. Nematopathogenic fungi

Fungi that infect and kill nematodes (worms) are referred to as nematopathogenic fungi. Over 150 species of fungi are known to invade nematodes. Nematode-destroying fungi can be grouped into three: nematode-trapping fungi, the endoparasitic fungi and the fungal parasites of cyst and root-knot nematodes. Most nematopathogenic fungi fall in the group of nematode trapping; they use constricting (active) or non-constricting (inactive) rings, sticky hyphae, sticky knobs, sticky branches or sticky networks at intervals along the length of a widely distributed vegetative hyphal system to trap and kill nematodes by penetration and growth of hyphal elements within the host, for example, *Arthrobotrys candida, A. oligospora, Drechmeria coniospora* [58], *Harposporium anguillulae* [59] *and Monacrosporium* spp. [60]. The nematode-trapping fungi, *Duddingtonia flagrans*, which have demonstrated considerable superiority in the reduction of gastrointestinal nematodes parasitizing animals, produce thick-walled clamydospores that enable it to survive the passage through the gastrointestinal tract and is therefore effective in destroying the larval stages of parasitic nematodes in livestock [61, 62]. Feeding or field trials have clearly demonstrated that dosing with a few hundred thousand spores per kilogram of live birth weight (BW) of *D. flagrans* not only reduced the number of infective larvae but also increased the BW of the lambs compared with controls [63]. In another example, Araujo et al. [64] tested the nematode-trapping fungus *Arthrobotrys robusta* against *Cooperia punctate* larvae (L3) and observed a 53.81% reduction in the helminths eggs (EPG) in treated calves compared to non-treated calves as well as a 70.45% reduction in the number of recovered worms at necropsy in the treated calves compared to the control. Endoparasitic fungi infect nematodes by spores, which then develop and absorb the body contents, for example, *Harposporium anguillulae* [65]; meanwhile, the fungal parasites of cysts and root-knot nematodes exert their effect by invading eggs or females by ingrowth of vegetative hyphae, for example, *Verticillium chlamydosporum* [66–69]. Nematode-trapping fungi have increasingly been tested in the management of parasitic nematode infections of ruminants [70].

5.3.2. Bacteria

The most important entomopathogenic bacteria belong to the genera of *Bacillus* (see **Figure 1**). *B. thuringiensis* (Bt) is among the most widely used antagonist in the biological control of insects. After ingestion, target insects are killed by an enterotoxin released from a crystal protein in the bacterial spores. The mode of action of the toxin has been fully described [46]. Various subspecies of Bt has been used in biocontrol: *Bacillus thuringiensis* var *israelensis* (Bti), with activity against mosquito larvae, blackfly (Simuliid), sand fly, fungus gnats and related dipterans species; *B. thuringiensis* var *kurstaki* (Btk) and *B. thuringiensis* var *aizawai* (Bta) with activity against lepidopteran larval species; *B. thuringiensis* var *tenebrionis*

(Btt) with activity against coleopteran adults and larvae and *B. thuringiensis* var *japonensis* (Btj) strain buibui, with activity against soil-inhabiting beetles [46]. In some countries, commercial formulation of *B. thuringiensis var israelensis* is available for the control of mosquito larvae and the blackfly *Simulium damnosum* [71]. A study performed in Egypt comparing the activity of three *Bacillus thuringiensin* products in controlling ticks shows that Btk was more potent compared to Bti and *Bacillus thuringiensin* var *thuringiensin* in the control of ticks [72]. Several products of *Bacillus thuringiensin* are available in the market; a few examples of products include Dipel 2x (*B. thuringiensis* var. *kurstaki*), VectoBac (*B. thuringiensis* var. *israeliensis*) and HD 703 (*B. thuringiensis* var. *thuringiensis*) [72]. VectoBac, Bti (12 AS, Wady El Niel for agricultural development Co. Egypt) has been shown to be highly effective against *C. pipence* than *M. domestica* [53].

Mosquito larvae are also susceptible to *B. sphaericus*. *B. sphaericus* is effective in killing larvae of *Culex* spp. and *Anopheles* spp., especially those breeding in polluted water. Bti and *B. sphaericus* have been reported to successfully control certain species of sand fly (vector for the protozoa *Leshmania*) [73]. *B. penetrans* is also a well-known nematopathogenic bacterium of plant parasitic root-knot nematodes.

The bacterial pathogen, *Paenibacillus glabratella,* recently discovered by Duval et al. [74] has been observed to infect and cause high mortality in snails, therefore, making a promising BCA for the control of snails.

Another bacterium, *Streptomyces avermitilis*, produces toxins collectively called "avermectins" which are highly effective against several invertebrates from the classes Insecta, Arachnida and Nematodes [21]. *Streptomyces griseolus* has been shown in the laboratory to be able to control the trematode liver fluke, *Fasciola gigantica*, the causative agent of Fasciolosis [75]. Streptomyces are believed to kill parasites by the production of lytic enzymes such as α and β-glucanases, proteases, peptidases, cellulases, chitinases and lipases [75].

Bacteria belonging to the following genera have been tested for the control of plant parasitic nematodes including *Pasteuria* which are parasites of many plant-parasitic nematodes and water fleas [76]; *Brevibacillus laterosporus* strain G4 which is parasitic on *Heterodera glycines*, *Trichostrongylus columbriformis* and *Bursaphelenchus xylophilys* and the saprophytic nematode *Panagrellus redivius* [77, 78]; rhizobacteria (mainly *Bacillus* spp. and *Pseudomonas* spp.) are able to antagonize nematodes [79, 80] and the well-studied *Bacillus thuringiensis* (Bt) are also able to kill plant-parasitic nematodes [81]. More information about using bacteria as biocontrol agents has been extensively reviewed by Khater [46] and Tian et al. [82].

Nota bene: The Rickettsiae are a diverse group of bacteria, which cause diseases to humans and warm-blooded animals, and are transmitted by a number of arthropods such as ticks, fleas and so on. Some of these bacteria tend to parasitize these arthropods [83]. For example, ticks have become adapted as vectors, reservoirs and/or propagation sites of Rickettsiae [84] and often harbour generalized asymptomatic infections. Rickettsial infection may lead to alterations in tick behaviour, interfere with their development and cause pathological changes in salivary glands and ovarian tissues. In severe cases, infection may lead to death [85]. However, the use of Rickettsiae in biocontrol is not a reliable method.

5.3.3. Viruses and virus-like particles

Thousands of entomopathogenic viruses have been described but only a few, belonging to the families Entomopoxviridae (Entomopoxviruses, EPVs), Reoviridae (Cypoviruses, CPVs) and Baculoviridae (Baculoviruses, BVs), have been used successfully in controlling pest population [86]. The mode of pathogenesis and replication of entomopathogenic viruses varies according to the family, but infection nearly always occurs by ingestion [46]. The baculovirus (see **Figure 1**) is the most widely exploited virus group for biocontrol [87, 88]; they are very different from viruses that infect vertebrates and are considered very safe to use. The family Baculoviridae contains four genera: Alphabaculovirus (lepidopteran-specific NPVs), Betabaculovirus (lepidopteran-specific GVs), Gammabaculovirus (hymenopteran-specific NPVs) and Deltabaculovirus (dipteran-specific NPVs) [89]. At present, there are approximately 16 biopesticides based on baculoviruses available for use or are under development. The majority of these products are targeted against Lepidoptera. For example, codling moth granulovirus, CpGV (Cydia pomonella Granulovirus), is an effective biopesticide of codling moth caterpillar pests of apples, Gemstar LC (NPV of *Heliothis/Helicoverpa* spp. e.g. corn earworm, tobacco budworm and cotton bollworm); Spod-X LC (NPV of *Spodoptera* spp. e.g. beet armyworm); CYD-X and Virosoft CP4 (GV of *Cydia pomonella*, the codling moth) and CLV LC (NPV of *Anagrapha falcipera*, the celery looper) [46].

The leafhopper-infecting virus, Homalodisca coagulate virus-1 (HoCV-1, Dicistroviridae), has been shown to increase leafhopper mortality [90, 91]. The virus occurs in nature and spreads most readily at high population densities through contact among infected individuals, contact with virus-contaminated surfaces and/or as an aerosol in leafhopper excreta.

5.3.4. Protozoa

Very little attention has been given to entomopathogenic protozoans. Some protozoa such as *haemogregarina*, *Nosema*, *Babesia* and *Theileria* are pathogenic to some arthropods like ticks. Although there are no examples of effective direct biocontrol of protozoans, however, indirectly, some protozoans such as *Plasmodium* spp. and *Onchocerca volvulus* may indirectly be controlled by their intermediate hosts or vectors. The predatory soil amoeba *Theratromyxa weberi* is capable of ingesting nematodes. It flows over the nematode body and assimilates it within 24 h. This and other amoebae can be expected to have limited biocontrol capacities because they are slow-moving, as compared to nematodes. Other protozoa including *Nosema locustae* are pathogenic to grasshoppers and crickets; *Nosema pyrausta* (also known as *Perexia pyraustae*) is pathogenic to the European corn borer and *Vairimorpha necatrix* occurs naturally and infects corn earworm, European corn borer, armyworms, fall webworm and cabbage looper [92].

5.3.5. Nematodes

Numerous nematodes belonging to the genera *Steinernema* and *Heterorhabditis* are either obligate or facultative parasites of insects (including houseflies, fleas and other non-biting flies) and have been proven as effective BCAs [86] to control a wide range of insect pests including filth flies, German cockroaches, cat fleas, armyworms, carpenter worms, crown borers, cutworms, flea beetles, leaf miners, mole crickets, plume moths, sciarid flies, root weevils, stem borers, webworms and so on [93]. They infect through penetration of the cuticle; invasion through the spiracles or anus or after ingestion by the host insect. The symbiotic bacteria contained within the nematode, when released into the body of the insect, cause septicaemia and death of the host. The bacteria then break down the insect body, which provides food for the nematodes. The nematode-bacterium relationship is highly specific; only *Xenorhabdus* spp. coexists with Steinermatids and only Photorhabdus bacteria coexist with heterorhabditids [46]. *Steinemema carpocapsae* (see **Figure 1**) has demonstrated effectiveness in the control of mosquitoes [49]. The host-specific entomoparasitic nematode, *Heterlylenchus autumnalis,* has been observed to parasitize *Musca autumnalis,* resulting in sterile female flies as nematode development occurs at the expense of egg production [94]. Entomopathogenic nematodes have been used commercially against insects during the last decades [95]. Seven species of nematodes have been commercialized worldwide and seven are currently available in the USA: *Steinernema carpocapsea*, *S. feltiae*, *S. glaseri*, *S. riobraus*, *Heterorhabditis bacteriophora*, *H. megidis* and *H. marelatus* [96]. There are two commercial nematode products available for termite control, Spear® and Saf T-Shield® [46].

The nematode *Paraiotonchium muscadomesticae* is also known to infect housefly larvae, but mortality is usually low except at high nematode concentrations. *P. muscadomesticae* infects housefly larvae and its descendants invade and damage the ovaries of adult female flies and are deposited in the larval habitat when the flies attempt to oviposit [46]. Furthermore, it has been observed that infected adults lived only about half as long as uninfected adults [97]. All these indirectly reduce housefly population.

Steinernema carpocapsae and *S. glaseri* have also been proven to be effective against *Teladorsagia* spp. and *Trichonstrongylus* spp. These nematodes are particularly useful on ground-inhabiting stages of fleas [98] and engorged females of numerous other ticks that fall to the ground [99–101]. Entomopathogenic nematodes are not harmful to humans, animals or plants and are generally regarded as remarkably safe to the environment [46].

Chaetogaster limnaei (Oligochaeta: Naididae) has long been observed to infect freshwater snails and protect the host from infection with various species of trematodes by eating both the miracidia and the cercaria. *C. limnaei* therefore has potentials in the biocontrol of parasitic diseases vectored by the freshwater snail (such as fascioliasis, schistosomiasis and related trematode infections) [102].

5.4. Earthworms

Earthworm population consume a large volume of soil and organic matter such as animal faeces. During feeding, they consume nematodes present in the soil and faeces. In different parts of the world, earthworms are responsible for natural biological control of trichostrongyle nematodes. For example, in northern Europe, earthworms play an important and often dominating role in removal of cattle dung from pastures and can be responsible for significant reduction of infective larvae of trichostrongyle nematodes on the pasture [103].

For more information about the life cycle, safety, production and genetically modified organisms (GMOs) of biological control agents, see Khater [46].

6. Effect of biological control on the native biodiversity

Biocontrol may have potential positive or negative effects on the diversity of native species. One of the major problems with biocontrol is the effect of the BCA on non-target species; the purpose of introduction of a BCA is to reduce the competitive advantage of exotic species that has previously invaded or been introduced there over the native species. However, the introduced BCA does not always target only the intended species; it can also target native species. Therefore, when introducing a BCA to a new area, a primary concern is its host specificity. BCA not targeting one species or a narrow range of species often makes for poor BCA and may become invasive species themselves. For this reason, potential BCA should be subject to extensive testing and quarantine before release to any environment. If an introduced species attacks the native species, this can lead to widespread changes in the biodiversity in that area. A classic example of biocontrol gone wrong is the cane toad that was introduced in Australia to control the introduced French's cane beetle and the Greyback cane beetle [104]. The toad instead was feeding on the native insect and soon took over native amphibian habitat and brought foreign disease to native toads and frogs, dramatically reducing their population.

Another notable example where biocontrol has gone wrong is in the introduction of the small Asian mongoose (*Herpestus javanicus*) found in the wild in South and Southeast Asia to Hawaii [105, 106]. *H. javanicus* was introduced to Hawaii over 100 years ago to control the rat population that were destroying the sugarcane plantations. Mongooses are entirely diurnal [107] meanwhile rats are nocturnal. The mongoose preyed on the endemic birds, especially their eggs, more often that it preyed on the rats. Both the mongoose and the rats now constitute a major threat to the bird's population in Hawaii. A few attempts have been made to eradicate these invasive mongooses but with limited success [108], and now the small Asian mongoose is regarded as one of the world's 100 worst invasive species [108, 109].

Furthermore, the sturdy and prolific eastern mosquitofish (*Gambusia holbrooki*), native to the eastern and southern United States, was introduced around the world in the 1930s and 1940s to feed on mosquito larvae and thus combat malaria. Unfortunately, it has thrived at the expense of local species, causing a decline of endemic fish and frogs through competition for food resources as well as through eating their eggs and larvae [110]. Many characteristics have been identified in Gambusia that contribute to their invasiveness: mosquitofish have short breeding periods and high fecundity [111], they exhibit higher feeding rates than their non-invasive relatives [112] and also show evidence of plastic responses to salinity-related stress; they produce more offsprings in higher salinities [113].

On the other hand, the replacement of the target species with another species which constitute more of a nuisance and for which the BCA does not normally attack is another challenge of biocontrol. This has happened in the past, for example in Douglas county, Oregon, USA, where Klamath weed populations were sharply reduced by biocontrol agents only to be

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replaced by tansy ragwort (*Senecio jacobaea*), which was in turn sharply reduced by BCAs only to be replaced by the Italian thistles (*Carduus pycnocepahalus*) [114].

7. Approaches to biocontrol

There are three broad approaches to biocontrol.

7.1. Importation

Importation involves the importation, screening and release of natural enemies to permanently establish effective natural enemies in a new area. Importation (also referred to as "classical biological control") usually targets introduced (non-native) pests in an area where their natural enemies normally do not exist. Native pests that are not adequately controlled by existing natural enemies may also be the target of classical biocontrol. The introduction of natural enemies to control the population of a pest is usually tightly regulated and is conducted solely by the federal or state agencies compared to the following two approaches that can be done by anyone [115]. This is necessary so that we do not import "solutions" that become more serious than the "problems" themselves.

7.2. Augmentation

Augmentative biological control typically involves the purchase and release of natural enemies that are already present in an area but not in quantity, enough to adequately keep in check the pest population in a particular location. The goal of this approach is simply to increase the number of natural enemies temporarily and therefore decrease the pest population in the area [115].

Release of natural enemies may take one of these forms: inundation or inoculation. With inundation, the target area is flooded with a large number of the natural enemies. Ideally, such a release will bring the pest(s) under control quickly, and it is hoped that the natural enemies will become permanently established in the area. Meanwhile, inoculation of an area usually involves much lower numbers. It is designed to allow establishment of a biological control agent in an area. Or such a release may be used merely to improve the natural enemy/pest ratio [116].

7.3. Conservation

This involves practices to conserve the population of natural enemies, thereby improving their effectiveness in the control of pests. Such practices include farming and gardening that provide the necessary resources for their survival and protect them from toxins and other adverse conditions. These conservative practices will benefit all natural enemies, whether native or imported or released through augmentation. This approach is frequently overlooked, yet it is just as important as the other two approaches [115].

8. Case studies of biocontrol

One of the most foretold stories of the success of biocontrol on a large scale is the eradication of the cottony cushion scale (origin: Australia) which was a serious threat to the citrus industry. The cottony cushion scale (*Icerya purchasi* Maskell) was introduced into North America and India and it rapidly spread, threatening the citrus crop. Chemical methods were either ineffective or too expensive to control the pest. The coccinellid beetle, *Rodolia cardinalis*, which is the natural enemy of the scale in its native Australia was introduced into the areas affected by the scale in North America and India, and it successfully controlled the cottony cushion scale [5]. Since then, other successes on a similar scale have been recorded, most in the biocontrol of parasites (pests) of plants. Biocontrol of parasites of medical and veterinary importance is still at its infancy, that is at the level of research, and as such no success story has been described. A few success stories in the biocontrol of parasites of agricultural importance are described below.

8.1. Case study 1: biocontrol of water hyacinth *(Eichhornia crassipes)*

Water hyacinth is a free-floating aquatic weed of South American origin and ranks among the top 10 weeds worldwide. It is one of the most noxious weeds known to man and has spread to at least 50 countries around the globe. The weed grows and occupies water surfaces of ponds, tanks, lakes, reservoirs, streams, rivers and irrigation channels. It was also a menace in flooded rice fields, considerably reducing yield. It interferes with the production of hydroelectricity, blocks water flow in irrigation channels, prevents the free movement of navigation vessels, interferes with fishing and fish culture and facilitates breeding of mosquitoes as well as fostering waterborne diseases [117]. Furthermore, water loss due to evapo-transpiration was a major concern especially in areas where freshwater shortage was common. Under ideal conditions, water hyacinth plants can propagate vegetatively and double their number in 10 days; the seeds can remain dormant for as long as 20 years before germinating [117]. The weed was indeed a major problem in India [117]. With this high growth rate, the weed defied most control methods.

Three exotic natural enemies were introduced in India, that is hydrophilic weevils—*Neochetina bruchi* (from Argentina) and *N. eichhorniae* (from Argentina)—and galumnid mite *Orthogalumna terebrantis* (from South America) in 1982 for the biological suppression of water hyacinth. Starting from October 1983, field releases of mass-bred weevils *N. eichhorniae* and *N. bruchi* in different water tanks in Karnataka, located at Byramangala (500 ha), Bellandur (344 ha), Varthuru (40 ha), Hebbal (20 ha), Nagavara (20 ha), Agram (20 ha) and others from October 1983 to December 1986; in an 8-ha tank at Nacharam in Hyderabad (Andhra Pradesh) in 1987; Ramgarh lake near Gorakhpur (Uttar Pradesh) in 1988; in 43-km-peripheral Surha lake, Balia, (Uttar Pradesh) in 1990 and Lakhaibill (Alengmore) and Assam in 2000, resulted in suppression of water hyacinth within 4 years. The weevils have cleared the Tocklai River and were proving very effective in most of the water bodies. Releases of the water hyacinth mites, like *O. terebrantis,* which are specific to water hyacinth were initiated in 1986 at Bangalore, Karnataka. About 25,000 adults were released in Agram, Kengeri and Byramangala tanks.

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Establishment was obtained within 6 months in all the tanks. The mite was more efficient in water in which *N. eichhorniae* was also present [117]. Many other successes have been recorded in the biocontrol of weeds which can be found in the review by Cork et al. [118].

8.2. Case study 2: biocontrol of the glassy-winged sharpshooter *(Homalodisca virepennis* formerly known as *H. coagulata)*

The glassy-winged sharpshooter (*Homalodisca vitripennis*) is a large leafhopper insect from the family Cicadellidae. It is native to North America (northeastern Mexico) but has spread into the USA, where it has become an agricultural pest [119]. It is thought that the glassy-winged sharpshooter invaded and was established in the southern California sometimes around 1990 [120]. The glassy-winged sharpshooter usually lays a mass of eggs on the underside of leaves and covers them with the "brochosomes", a powdery white protective secretion kept in dry form. After hatching, the nymphs feed within the vascular system of small stems, molt several times and become adults which continue to feed on a wide variety of plants including grapes, citrus trees, almonds, stone fruit and oleanders resulting in enormous damage. Their feeding method along with their voracious appetite for so many different hosts makes them an effective vector for the bacterium *Xylella fastidiosa*, which causes plant disease. *X. fastidiosa* has been linked to many plant diseases including phoney peach disease in the southern USA; oleander leaf scorch and Pierce's disease in California and citrus X disease in Brazil. Plants not affected by the bacterium become a reservoir for other sharpshooters to pick up and carry to other plants [121].

The glassy-winged sharpshooter has a number of natural enemies, in particular egg parasitoids. Female parasitoids lay their eggs inside glassy-winged sharpshooter eggs and the developing parasitoid larvae kill glassy-winged sharpshooter eggs by feeding inside the egg. The parasitoid larvae pupate inside the glassy-winged sharpshooter egg and then chew a circular exit hole through which they emerge. The winged parasitoids can fly and after mating, they look out for more glassy-winged sharpshooter eggs to parasitize. In this manner, the egg parasitoids help keep the glassy-winged sharpshooter population in check [121]. In the southeastern USA and northeastern Mexico, glassy-winged sharpshooter eggs are parasitized by several species of mymarid and trichogrammatid parasitoids, including *Gonatocerus ashmeadi* Girault, *G. triguttatus* Girault, *G. morrilli* Howard and *G. fasciatus* Girault. Virtually, all species of parasitoid in the family Mymaridae (order: Hymenoptera) are the most common natural enemies associated with *H. vitripennis* eggs in the southeastern United States [122]. *Gonatocerus tuberculifemur* and *G. deleoni* are other species of parasitoids that attack the glassy-winged sharpshooter eggs and were introduced into California from Argentina [123, 124].

The glassy-winged sharpshooter has also successfully invaded French Polynesia (the Society Islands, Marquesas and Austral Island groups) where it became established in 1999 [125], Hawaii where it became established in 2004 [126], Easter Island and the Cook Islands. Glassy-winged sharpshooter became established in Tahiti French Polynesia in 1999 and was likely introduced accidentally on ornamental plants imported from California [121]. In contrast to California, no natural enemies for the glassy-winged sharpshooter existed there, and no obvious competitors existed in urban or natural settings. The glassy-winged sharpshooter populations underwent an exponential growth and were a complete nuisance to the population;

watery excreta known as sharpshooter rain literally rained from infested trees because there were so many glassy-winged sharpshooters feeding on trees, their noisy wings, their dead bodies littered in houses and their high populations retarded plant growth and reduced local fruit production [121]. Due to intense population movement, the glassy-winged sharpshooter spread to other areas in the region such as Raiatea (Leeward Islands) Moorea, Leeward Islands of Huahine, Bora Bora, Tahaa and Maupiti. At the end of 2004 and the beginning of 2005, the glassy-winged sharpshooter populations were discovered outside of the Society Islands in two other archipelagos of French Polynesia substantially distant from Tahiti: the Australs, where two islands were infested (Rurutu and Tubuai) and the Marquesas, where one island, Nuku Hiva, was found infested [121].

To combat the glassy-winged sharpshooter infestation in French Polynesia, the mymarid egg parasitoid *Gonatocerus ashmeadi* was imported from California, mass bred and released. Between May and October 2005, 13,786 parasitoids were released at 27 sites in Tahiti. The parasitoid established readily, and within 7 months of release, the glassy-winged sharpshooter had been completely controlled in Tahiti, and glassy-winged sharpshooter populations were reduced by over 95% [127]. The parasitoid also spread unassisted to every other island infested by the glassy-winged sharpshooter and parasitized their eggs [121], which led to a successful control of the glassy-winged sharpshooter in the area.

8.3. Case study 3: biocontrol of the velvet bean caterpillar (*Anticarsia gemmatalis*)

One of the most successful uses of baculoviruses in biological control has been in Brazil. The baculovirus AgMNPV has been successfully used in the control of the velvet bean caterpillar (*Anticarsia gemmatalis*), a pest of soybeans. Plots of soybeans that were naturally infested with *A. gemmatalis* were sprayed with the virus. The AgMNPV is highly virulent for *A. gemmatalis* and only needs to be applied once, which makes it a good BCA for the control of the velvet bean caterpillar. Furthermore, the virus can be spread by insect predators and survive passage through the digestive tract of beetles and hemipteran. In Brazil, virus preparations were applied at 1.5×10^{11} occlusion bodies per ha, that is, about 20 g or 50 larval equivalents. The programme, which was initiated in the early 1980s, by 2005, had seen the treatment of area of over 2 m ha [128].

In other examples, the granulovirus of the codling moth *Cydia pomonella* (CpGV) has been used in a number of countries in North America and Europe for the control of the insect on pear and apple crops [128]. In China, the baculovirus HearNPV has also been successfully used to control the cotton bollworm, *Helicoverpa armigera*, which was a major pest of cotton and had developed resistance to the chemical insecticides in many parts of the world [129].

9. Challenges in the biological control of parasites

Some challenges in the implementation of biocontrol strategies are listed below.

The introduction of exotic natural enemies raises concern regarding the effect it may have on non-target native species as mentioned above. Conservation biologists are typically concerned with the health and growth of a wide variety of organisms. If a BCA does in fact attack any native non-target species, its persistence and ability to spread to areas far from the site of release become a serious liability [130–132].

There are also concerns among conservation biologists about the release of BCA precisely because the agents themselves which are non-native may carry non-native parasites and commensal species [114].

BCAs are easily influenced by environmental factors such as temperature, humidity and oxygen extremes, which determine the success of the biological control strategy. BCA if applied when conditions are not favourable is bound to fail.

There are also challenges in the distribution of BCAs product, especially those containing living organisms. Most industries producing BCA products are often situated a considerable distance away from where the BCA is to be used. Before the BCA reaches its destination, most of the organisms are dead. There is therefore the need to develop a sizeable distribution network comprising a group of producers that will safeguard the quality of the products and provide advice for the users [133].

Another challenge, which may be faced with the implementation of a biocontrol strategy in pest control, is the lukewarm attitude among agriculturalists, who find it difficult to forego their fast-acting chemical pesticides over the sluggish BCA [134, 135].

10. Future perspective

10.1. Biotechnology

With the advances in biotechnology, there is the potential of identifying and manipulating "biocontrol genes" particularly in microbial agents to produce more effective BCAs. Furthermore, genes in BCAs responsible for their antagonistic effects will also be used to screen for more effective BCAs. Biotechnologists in many countries are experimenting with fungi, viruses, bacteria, nematodes and insects genetically modified to express toxins (scorpion toxin, mite toxin and trypsin inhibitor), hormones (eclosion hormone and diuretic hormone) or metabolic enzymes (juvenile hormone esterase) to increase the speed of killing, enhance virulent and extend host specificity of these organisms. The so-called third-generation genetically modified organisms (GMOs) have been engineered to control pests in agriculture, pathogens in human health and invasive species in the environment [136].

In one approach, to improve the efficacy of *Bacillus thuringiensis* var *israelensis* (Bti), genes encoding the potent insecticidal proteins from Bti, Btj and *B. sphaericus* have been spliced into new bacteria strains that are 10-fold more toxic than wild types species of Bti and *B. sphaericus* used in current commercial formulations. These new GMOs are safe to humans, animals and the environment and can be used as components in the integrated vector control programmes aimed at reducing malaria, filariasis and other diseases of medical importance [137]. These recombinant bacterial larvicides are much more efficient than the wild-type strains from which they are derived, their costs are similar to the new chemical insecticides and they are much more environmentally compatible than most chemical insecticides [46].

In another approach, biotechnologists are trying to develop new biopesticides based on fungi. Fungi tend to be host specific, can be mass produced on inexpensive media and are thought to be harmless to animals, humans and the environment. Unfortunately, naturally occurring fungi tend to kill insects slowly. Genetic technology holds the promise of producing biopesticides based on hypervirulent insect-specific fungi that kill quickly; for example, laboratory experiments have been performed where scorpion toxin gene has been spliced into the fungus that infect mosquitoes to enhance the killing efficiency of the fungus [138].

In yet another approach, biotechnologists are studying baculoviruses, a large variety of viruses that act specifically on hundreds of arthropods, including many agricultural pests, but appear to be safe to plants and vertebrates. But because baculoviruses typically kill much more slowly than chemical pesticides, their use is limited. Biotechnologists are experimenting to increase the killing efficiency of baculoviruses by splicing into them toxin-expressing genes isolated from mites, scorpions and spiders [139]. Baculovirus recombinants that produce occlusion bodies incorporating Bt toxin have also been constructed by making a fusion protein consisting of a polyhedron and Bt toxin [140]. Other constructs have been tested with varying success [141]. This new biopesticide is highly pathogenic than the wild-type baculovirus as it combines the advantages of the virus and the bacteria toxin.

However, the use of biotechnology raises some questions regarding the potential impact of those GMOs or plants to human, animal and the environment and other non-target species. This has presented a major hurdle to research and field testing and the introduction of these recombinant BCAs to users. Fortunately, the use of genetically engineered microbial pathogen products for control is increasingly being accepted by the society, and commercial production is gradually gaining grounds. In the near future, genetically engineered microbial BCAs will soon be the most common biocontrol products available in the market to circumvent the problem of growing resistance to chemical pesticides and the threats posed to public health and the environment by the chemical pesticides.

10.2. Nanotechnology

Nanotechnology is another field that holds wide applicability in biological control in the near future. Nanotechnology for control has been applied mostly in the control of agricultural pests. Its application in the control of agriculture pest offers some advantages over traditional methods by providing green and highly efficient alternatives for the management of insect pests without harming nature [142]. Nanoparticles are known to be effective against plant pathogens, insects and pests. Hence, nanoparticles can be used in the preparation of new formulations like pesticides, insecticides and insect repellents [143–146]. Nanomaterials come in many forms—porous hollow silica nanoparticles (PHSNs) loaded with validamycin (pesticide) [147], nano-silica prepared from silica, polyethylene glycol-coated nanoparticles loaded with garlic essential oil, silver nanoparticles synthesized from various plant extracts and so on.

One of the most studied nanomaterials for the control of agricultural pests is nano-silica. Nano-silica formulated as nano-pesticide can effectively be used in the control of insect pests. The mechanism of control of insect pests using nano-silica is based on the fact that insect pests use a variety of cuticular lipids to protect their water barrier and thereby prevent death from desiccation. But nano-silica gets absorbed into the cuticular lipids by physiosorption and thereby causes death of insects purely by physical means when applied on leaves and stem surfaces [142]. It has also been shown that in addition to agricultural insect pests, surface-charged modified hydrophobic nano-silica (~3–5 nm) could be successfully used to control animal ectoparasites of veterinary importance [148].

Silver nanoparticles (AgNPs) have been synthesized using various plant extracts as reducing and stabilizing agents. These AgNPs have been tested and shown to be of higher toxicity against the mosquito vectors of parasites of medical and veterinary importance. For example, an AgNP synthesized using extracts of *Artemisia vulgaris* leaves has been observed to be highly toxic to *Aedes aegypti* larval instars (I–IV) and pupae, with LC_{50} ranging from 4.4 (for the first instar) to 13.1 ppm (for the pupae) and was also observed to increase the predatory efficiency of the Asian bullfrog tadpole, *Hoplobatrachus tigerinus*, a natural predator on mosquito larvae [149]. AgNP synthesized using other plant extracts has also demonstrated similar or higher efficacy: AgNP synthesized using the aqueous leaf extract of the seaweed, *Hypnea musciformis*, has shown larvicidal and pupicidal toxicity against *Aedes aegypti* and the cabbage pest, *Plutella xylostella* [150]; AgNP synthesized using *Nicondra physalodes* has shown larvicidal toxicity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, with the maximum efficacy detected against *An. Stephensi* (LC_{50} = 12.39 μg/ml) [151] and AgNPs synthesized using *Zornia diphylla* have shown higher toxicity against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaniorhynchus* with LC_{50} values of 12.53, 13.42 and 14.61 μg/ml, respectively [152]. AgNPs are promising for the development of eco-friendly larvicides against mosquito vectors, with negligible effect against non-target species.

Yang et al. [153] have demonstrated that the efficacy of the insecticidal activity of polytherlene glycol-coated nanoparticles loaded with garlic essential oil against adult red flour beetle (*Tribolium castaneum*) insects found in store products was as high as 80%. In another example, Sabbour [154] tested two nanomaterials Aluminium oxide (Al_2O_3) and Titanium dioxide (TiO_2) against rice weevil *Sitophilus oryzae* and observed that under laboratory conditions, the mortality increased significantly to 50.6 ± 3.6 as compared to 3.0 ± 3.4 for the control and under store condition, the mortality increased significantly to 67.3 ± 1.4 after 45 days of storage as compared to 3.8 ± 3.8 in the control. Furthermore, accumulative mortality (%) of *S. oryzae* beetles increased gradually by increasing the period of exposure.

Nanotechnology has also been applied on BCAs. Nanoparticles as various formulations of essential oils, silica gels, powders and so on applied on BCAs have been shown to increase the effectiveness of BCAs in neutralizing some agricultural pests. For example, Sabbour [155] showed that in the laboratory, the nano-entomopathogenic fungi, nano-*Beauveria bassiana* and nano-*Metarhizium anisopliae,* formulated using dust carriers, were more effective in the killing of the insect pest of stored rice *Sitophilus oryzae* (L.) compared to control. The LC_{50} obtained were 45×10^4 and 57×10^4 conidia/ml for nano-*B. bassiana* and nano-*M. anisopliae,* respectively, lower than 66×10^4 and 77×10^4 conidia/ml for *B. bassiana* and *M. anisopliae,* respectively. There was a significant reduction of the number eggs laid/female as well as the number of emerged adults in stored bags that were treated with nano-entomopathogenic fungi nano-*B. bassiana* and nano-*M. anisopliae* compared to control. On the other hand, some BCAs are capable of producing nanoparticles. For example, there are a large volume of research reports that suggest that actinobacteria are capable of producing metal oxide nanoparticles. These can be exploited in the green synthesis of nanomaterials and utilized in biological systems [156]. Sabbour [157] tested the efficacy of nano-extracted destruxin from *Metarhizium anisopliae* against the Indian meal moth *Plodia interpunctella* (which is one of the most serious stored grain pest worldwide), and the LC_{50} obtained was 77×10^4 for nano-destruxin compared to 103×10^4 for destruxin. Under laboratory conditions, the number of eggs laid/female significantly decreased to 17.4 ± 3.8 and 10.6 ± 9.5 eggs/female after treatment with destruxin and nano-destruxin as compared to 99.9 ± 7.9 eggs/female for the control after 120 days. And under store conditions, the number of eggs laid/female decreased significantly to 13.1 ± 9.2 after nano-destruxin treatments after 120 days. Furthermore, the emerged adults decreased to 2% after nano-destruxin treatments after 120 days [157].

Nanotechnology also has promising applications in nanoparticle-mediated gene (DNA) transfer. It can be used to deliver DNA and other desired chemicals into plant tissue for protection of host plants against insect pests [158]. There is evidence that nanotechnology will revolutionize agriculture including pest management in the near future [159].

10.3. Microencapsulation

Microencapsulation is another new field that holds promise in biological control. Microencapsulation is a process in which active substances are coated by extremely small capsules [160]. Microencapsulation has numerous applications in areas such as the pharmaceutical, agricultural, medical and food industries, being widely used in the encapsulation of essential oils, colourings, flavourings, sweeteners and microorganisms, among others [161]. Microencapsulation in biological control can be used for the enhancement of the activity of BCAs in biocontrol, especially pathogens. The coating may impact stability, protection from UV radiation and/or other environmental conditions, enhance the attractiveness of the pesticide to the pest and/or serve to separate two different biologically incompatible pesticides within a mixture. For example, *Bacillus subtilis* has been widely used as a BCA in agriculture but their short shelf life limits their use. In a study in which *Bacillus subtilis* was microencapsulated using maltodextrin, it was observed that the mean survival rate of *B. subtilis* was more than 90%, when spray drying was performed at 145°C, with a feed flow rate of 550 mL h−1 and a spray pressure of 0.15 MPa. The shelf life was also significantly prolonged compared to wettable powders. Moreover, the biocontrol efficacy of the *B. subtilis* microcapsule reached 79.91% when a dosage of 300 g hm^{-2} was used; the microcapsule showed higher control efficacy than thiram wettable powder against the plant pathogenic fungus *Rhizoctonia solani* in tomato under field conditions [162]. This approach can be applied to other BCAs, especially pathogenic microorganisms, to enhance their effectiveness.

11. Conclusion

To date, many strategies have been used in the control of parasites including the use of chemicals. The chemical methods are limited in their application, partly as a result of the rising

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resistance, environmental and health risks and the potential effect to non-target organisms. In addition to the previously mentioned biological control agents, parasites could also be controlled naturally through botanicals [163–167], photosensitizers [168, 169], symbiotic [170], organic [171] and short-chain fatty acids [172]. Biological control approaches hold promise as the most suitable alternative to the chemical pesticides and are now a core component of IPM. A good number of promising BCAs including predators, parasites (parasitoids) and pathogens (fungi, bacteria, viruses and virus-like particles, protozoa and nematodes) have been identified and proven to be efficacious against many parasites of medical, veterinary and agricultural importance, as highlighted in the chapter [25, 49, 85]. In the past, biological control has been applied successfully to control parasites especially in the agricultural sector [120]. However, there are still many challenges in the implementation of biological control strategies including their potential effects on native biodiversity [133–135], the unwillingness to ditch the chemical methods for BCAs by farmers [129] and challenges in the production and distribution of the BCAs [136]. With the recent advances in biotechnology and the application of most recent technologies such as nanotechnology [145] and microencapsulation [162], there are many opportunities for the continued use and expanded role of natural enemies in biological control; newer BCAs are being identified and older ones are being genetically engineered to make them more efficacious in their antagonism of parasites. There is, therefore, optimism that in the future, biological control will develop to overcome many of the challenges, and BCAs will become the mainstay for the control of parasites.

Abbreviations

IPM	Integrated pest management
BCA	Biological control agent
USDA	The US Department of Agriculture
Bt	*Bacillus thuringiensis*
BV	Baculovirus
GMO	Genetically modified organism
PHSNs	Porous hollow silica nanoparticles
DNA	Deoxyribonucleic acid
AgNPs	Silver nanoparticles
BW	Birth weight

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