i TECH MAG

DOI: http://doi.org/10.26480/itechmag.03.2021.19.26

ISSN: 2710-5873 (Online)
CODEN: ITMNBH

S&T REVIEW

AN OVERVIEW ON BIOLOGICAL CONTROL OF INSECT PESTS: REVIEW ARTICLE

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ARTICLE DETAILS

Article History:

Received 06 July 2021
Accepted 10 August 2021
Available online 12 September 2021

ABSTRACT

Natural ways of biocontrol are the best option to combat insect pests for ensuring maximum protection against crop failure. Since Nepal is blessed with natural resources, a huge number of biological control agents are available for the exploration of possible management of insect pests without causing any deleterious effects on the environment. They are less toxic, stable, and of no side effects when used in crop fields. The present generation of farmers is in dire need of sustainable biocontrol strategies that ensure optimum crop protection against harmful pests. This paper emphasizes various such biocontrol options that are capable of checking the pest population to prevent farmers from going through economic loss due to crop failure. Control measures with direct natural origin must be preferred over chemically synthesized ones if we hope to leave this Earth for our future generations as well. Researches on this field are still scanty. But with the healthy collaboration of government, research bodies ad local farmers, they could lead to the ultimate solution of insect pests through biological control methods soon.

KEYWORDS

Biocontrol, BCA, Insect- pest, Natural enemies.

1. INTRODUCTION

Insect pests have been dreadful woes to farmers for many decades leading to huge economic loss, and even total crop destruction in severe cases. Farmers are often in need of management strategies against insect pests to either control their population or prevent their future outbreaks. One of such oldest control measures is the biological control of insect pests, also known as bio-control (Savita & Sharma, 2019). Biological control is a population phenomenon, that is obtained from the interaction of a natural enemy population with the host population (N. J. Mills & Getz, 1996). More narrowly, biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease-resistant host plants, to suppress the activities and populations of one or more plant pathogens (Thomashow, 1996). These control measures are characterized by a persistent, strong reduction in the pest population following the introduction of a natural enemy (Beddington et al., 1978). The biotic organisms that are utilized for the suppression of insect pests are called Biological Control Agents (BCAs) (N. J. Mills & Getz, 1996).The total number of BCAs that function as control measures are mentioned in BIOCAT2010. BIOCAT2010 is a simple spreadsheet database, which sources published literature up until 2010 to provide details of all BCAs and their source country (countries), target pest(s), introduction location (including crop system) and date, whether it became established and results (Kenis et al., 2017). The information provided from BIOCAT2010 is valuable to analyze the suitable BCAs to apply as per the requirement of the pests' situation. A closer study of BIOCAT2010 also assists to choose the most suitable approach of biocontrol among Classical Biological Control (CBC), Augmentation, and Conservation.

The various studies and researches have highlighted that Biocontrol methods are the safest and most effective control measure in long run. While applying Integrated Pests Management (IPM), these Biocontrol measures must be included with other methods. This paper includes various biological control measures and approaches that can be applied safely to prevent from surpassing the economic threshold level of the pest population. Nepal, a country with rich biodiversity, can procure maximum benefit by promoting biological control approaches. To fulfill increasing population demand for a timely and adequate supply of agricultural products, quick and efficient measures are required to check the pest population. The devastating effects caused due to excessive use of chemical pesticides have persuaded people to shift towards more natural options. But still, a huge number of Nepalese farmers rely on heavy use of chemical pesticides against the pest species. Even those farmers that value biocontrol measures over chemical and other methods are not capable of incorporating the BCAs due to their unavailability. They often lack the technical guidance to apply these natural options. Hence, this article attempts to shed light on the possible control strategies that are suitable in the Nepalese context. It will be helpful to researchers, farmers, local and national level policymakers to formulate plans and programs focusing on promoting the biological control of insect pests.

2. MATERIALS AND METHODS

This paper is prepared by analysing secondary data obtained from various national and international open access journals, books, newspaper articles, reports, conference proceedings and bulletins. Most recent and up-to-date information were collected and incorporated in the data to prepare a final writing of the article.

3. RESULTS AND DISCUSSIONS

3.1 General overview of biological control history of insect pests in world

The history of the use of Biological control agents dates back to as early as 200 AD. While there isn't much written documentation found, but texts in Talmud from 200 AD have mentioned the use of ant colonies against each other to check their populations. In 300 AD, augmentation of Asian weaver ants (*Oecophylla smaragdina F.)* was done in citrus trees of China to protect them from insects that feed on foliage. Various unrecorded use of

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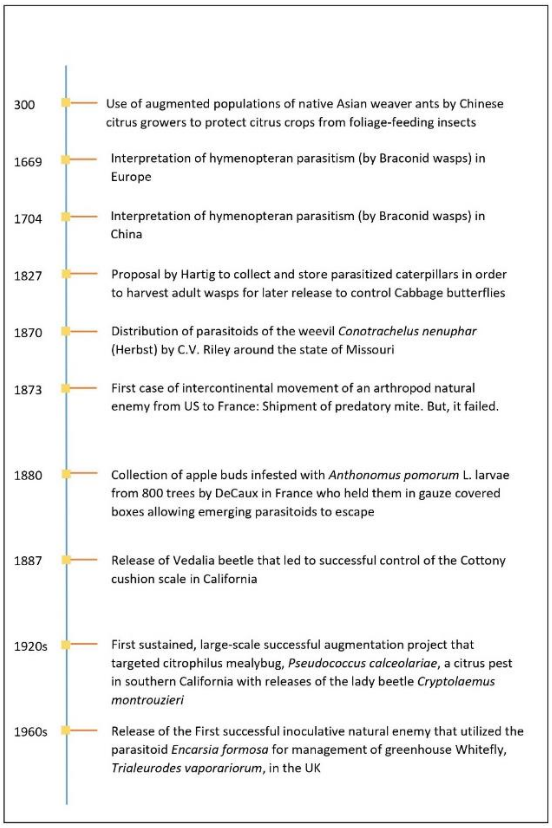
BCAs has been carried throughout centuries. The use of parasitized caterpillars to harvest adult wasps for later release to control Cabbage butterflies during 1827 and the distribution of parasitoids of the weevil (*Conotrachelus nenuphar)* by C.V. Riley around the state of Missouri were among some of the notable activities of controlling insect pest via biocontrol agents. The breakthrough in the progress of biocontrol methods took place in 1887 that included the control of the cottony cushion scale by another insect, the Vedalia beetle. The release of the beetles was successful to achieve complete control of the cottony cushion scale within two years (Orr & Lahiri, 2014). These advancements in biocontrol methods have paved the way for further initiatives. The general events on the history of biological control of insect pests is depicted in Figure 1.



300 Use of augmented populations of native Asian weaver ants by Chinese citrus growers to protect citrus crops from foliage-feeding insects

1669 Interpretation of hymenopteran parasitism (by Braconid wasps) in Europe

1704 Interpretation of hymenopteran parasitism (by Braconid wasps) in China

1827 Proposal by Hartig to collect and store parasitized caterpillars in order to harvest adult wasps for later release to control Cabbage butterflies

1870 Distribution of parasitoids of the weevil *Conotrachelus nenuphar* (Herbst) by C.V. Riley around the state of Missouri

1873 First case of intercontinental movement of an arthropod natural enemy from US to France: Shipment of predatory mite. But, it failed.

1880 Collection of apple buds infested with *Anthonomus pomorum* L. larvae from 800 trees by DeCaux in France who held them in gauze covered boxes allowing emerging parasitoids to escape

1887 Release of Vedalia beetle that led to successful control of the Cottony cushion scale in California

1920s First sustained, large-scale successful augmentation project that targeted citrophilus mealybug, *Pseudococcus calceolariae*, a citrus pest in southern California with releases of the lady beetle *Cryptolaemus montrouzieri*

1960s Release of the First successful inoculative natural enemy that utilized the parasitoid *Encarsia formosa* for management of greenhouse Whitefly, *Trialeurodes vaporariorum*, in the UK

Figure 1

3.2 Biological Control Agents (BCAs)

Biological Control Agents or Biocontrol Agents (BCAs) are defined as those organisms found naturally or genetically engineered that would eliminate or control the pest population to prevent economic losses occurring due to harmful effects from the targeted pests (Mishra et al., 2020).

BCAs function through various mechanisms. They compete with targeted pests for food and area that reduces their chance of survival. Some agents release toxic substances against the pests which infect and even kills in some cases. Figure 2 demonstrates the mechanisms involved in the effects caused by BCAs (Mishra et al., 2020).

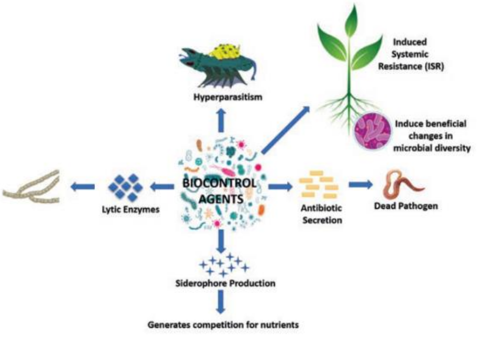


Figure 2: Mechanisms involved in Biological Control of pests.
Source: (Mishra et al., 2020)

Depending on the nature of origin if the BCAs, they are divided into classical agents and augmentation agents. The classical BCAs are introduced from a foreign location to control the pest population while the augmentation agents may or may not be available in the area of application, but inoculatory growth of introduced species is observed in the case of augmentation (Mishra et al., 2020). BCAs are being adopted as a superior alternative to the chemical control methods due to their minimum negative effects to the environment. BCAs can be also divided into predators, parasites and parasitoids, and pathogens depending on the organisms used as agents and their affecting mechanisms on pests.

3.2.1 Predators

Predators are the organisms that kill other species externally and feed upon them (Dreistadt, 2014). The species being killed are called prey. These predators are usually larger than the prey. Though predatory biocontrol agents are suitable to use as per environmental concerns, they aren't highly effective due to their generalist nature. So while choosing predator species, those with narrow host range and synchrony with prey's life cycle are to be selected. The predators should have efficient search ability and high reproductive potential. Some of the examples of predator BCAs that have had significant success are shown in the Table 1.

Table 1: Predator BCAs and their targeted with the locations that have had successful implementation

S.N.	Predator BCAs	Pests	Locations with successful control
1.	Tachinid fly *(Ptychomyia remota)*	Coconut moth (*Levuana iridescens*) Oriental moth (*Cnidocampa favescens*)	Fiji Massachusetts
2.	*Catana clauseni*	Citrus blackfly	Cuba, the Panama Canal Zone and Costa Rica
3.	*Cryptognatha nodiceps*	Coconut scales	Fiji
4.	*Chilocorus cacti*	Pustule scale (*Asterolecanium pustulans*)	Puerto Rico
5.	Lady Bird Beetle (*Coccinellidae*)	Aphids, Mealy bugs	
6.	Praying Mantid (*Mantodae*)	Moths, Crickets, Grasshoppers	
7.	Tiger beetle (*Cicindellidae*)	Gundi bug	
8.	Spiders	Aphids, Flea beetle, Red pumpkin beetle, Leaf hoppers	
9.	Water scorpion (*Nepidae*)	Mosquitoes	
10.	Syrphid flies	Aphids, Scale insects, Mealy bugs	

Source: (TAYLOR, 1955)

3.2.2 Parasites and Parasitoids

Parasites are the free living organisms that invade other organisms infecting their health, behaviour, population size, food web dynamics, and community structure (Raga et al., 2009). These parasites do not kill the host species, but live on or inside them deriving nutrients. Instead, parasitoids are the holometabolous insects that parasitize the host eventually. These parasitoids are highly host specific, and hence are one of the preferred agents of classical biological control. More than 3600 parasitioids have been introduced for the control of 500 arthropod species around the world killing (N. J. Mills & Getz, 1996). Some of the successful implementation of these parasites and parasitoids are shown in the Table 2 below:

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Table 2: Parasites and parasitoids as Bio-control agents with their host species and area with successful implementation

S.N.	Parasite and parasitoids BCAs	Pests	Locations with successful control
1.	*Ptychomyia*	*Artona catoxantha*	Malaya
2.	*Macrocentrus ancylivorus*	Oriental fruit moth	USA
3.	Egg parasitoids of *Trichogramma* spp	Codling moth (*Carpocapsa pomonella*)	USA, Spain, Russia
4.	Pupal parasites of *Pteromalus puparum*	Imported cabbageworm (*Pieris rapae*)	New Zealand
5.	Egg parasite of *Trichogramma minutum*	Sugarcane borer (*Diatraea saccharalis*)	Barbados, Peru, Puerto Rico
6.	*Mesoleius tenthredinis*	Larch sawfly (*Pristiphora erichsonii*)	Canada
7.	Larval and Pupal parasite of *Pleurotropis parvulus*	Coconut leaf-mining beetle	Fiji
8.	Egg parasite of *Anaphoidea nitens*	Eucalyptus snout weevil	South Africa
9.	*Opius tryoni*	Fruit flies (*Tephritidae*)	Mediterranean regions
10.	*Coccophagus gurneyi* and *Tetracnemus pretiosus*	Citrophilus mealybug	California
11.	*Eretmocerus serius*	Citrus blackfly	Cuba, the Panama Canal Zone and Costa Rica
12.	*Aphytis mytilaspidis*	Fig scale (*Lepidosaphes ulmi*)	Italy

Source: (TAYLOR, 1955)

The parasitoids can be divided into four categories ie egg parasitoids, larval parasitoids, pupal parasitoids and adult parasitoids on the basis of the life stage of the host species they attack on.

3.2.2.1 Egg parasitoids

Egg parasitoids are those parasitods that invade eggs of host species and killes them in the egg stage arresting them to hatch and advance into larval stage. These egg parasitoids are regarded highly effective as they kill the host species at very earlier stage before any crop loss has occurred. The BIOCAT database has provided 157 unique combinations of egg parasitoid amd host species, out of which 124 has known outcome (N. Mills, 2010).

A brilliant example of egg parasitoids to control pest biologically is the use of Trichogramma species. Trichogramma has been effective to check the pest population againststem borers in graminaceous crops and Helionthis/ Helicoverpa spp in corn, cotton, and tomato among others (N. Mills, 2010). Females of Trichogramma lay their eggs inside the insects' eggs that hatch and complete their larval and pupal stage, and emerges outside the host' egg after its complete destruction. One trichogramma species is enough for destroying 90-100 hosts' eggs (Rao, 2011).

3.2.2.2 Larval parasitoids

Larval parasitoids are those parasitoids that lay their eggs into or outside of larval hosts which hatch and feed on the larva to survive. When fully fed, the larval parasitoid spins a cocoon and pupates either externally or internally and then emerges as an adult to feed, mate, and continue the life cycle (Saleh et al., 2020). Some of the larval parasitoids with their host species are mentioned in the Table 3.

Table 3: Larval parasitoids and their targeted pest species

S.N.	Larval parasitoids	Host/Pest
1.	*Bracon hebetor*	Larvae of *Opisina arenosella*
2.	*Campoletis chloridae (Ichni)*	Larvae of *Heliothis armigera*
3.	*Microplitis demolitor*	Larvae of *Heliothis armigera*
4.	*Goniozus nephantidis*	Larvae of *Opisina arenosella*
5.	*Cotesia plutella*	Larvae of *Plutella xylostella*

Source: (Rao, 2011)

3.2.2.3 Pupal parasitoids

Pupal parasitoids are those parasitoids that lay their eggs into or outside of pupal cocoon of the hosts which hatch and feed on the pupa to survive. The eggs of the parasitoids develop inside the pupal cocoon and emerges out of it hence, destroying the cocoon. Some of the pupal parasitoids with their host species are mentioned in the Table 4.

Table 4: Pupal parasitoids and their targeted pest species

S.N.	Pupal Parasitoids	Hosts/ Pests
1.	*Tricholyga bomycis*	Pupa of silkworm
2.	*Tetrastichus Israeli*	Pupa of *Opisina arenosella*
3.	*Ichneumon promissorius*	Pupa of *Heliothis*
4.	*Xanthopimpla stemmator*	Pupa of corn borer in maize stem
5.	*Tetrastichus brontispae*	Pupa of fruit fly *Bactrocera* spp

Source: (Rao, 2011)

3.2.2.4 Adult parasitoids

Adult parasitoids are those parasitoids that lay their eggs into or outside of adult hosts which hatch and feed on the adults to survive. Adult parasitoids are very rare in nature. Some of the adult parasitoids with their host species are mentioned in the Table 5.

Table 5: Adult parasitoids and their targeted pest species

S.N.	Adult parasitoids	Hosts/Pests
1.	*Encarcia Formosa*	Adult of Cotton whitefly (*Bemisia tabaci*)
2.	*Aphelinus mali*	Adult of aphids
3.	*Epiricania melanoleuca*	Sugarcane leaf hopper (*Pyrilla perpusilla)*

Source: (Rao, 2011)

3.2.3 Pathogens/ Entomopathogens

Entomopathogens are microorganisms that are pathogenic to arthropods such as insects, mites, and ticks. These are used for the microbial control of pests which can be a critical part of integrated pest management (IPM) against several pests. Several species of naturally occurring bacteria, fungi, nematodes, protozoans, and viruses infect a variety of arthropod pests and play an important role in their management. Those used most widely are different subspecies of the bacterium, *Bacillus thuringiensis* (Bt), which constitute approximately 80% of the microbials used for insect control. Of the remaining 20%, a few baculoviruses have been successful as classical biological control agents, or have achieved moderate success as viral insecticides in several developing countries. Fungi, protozoa, and parasitic nematodes have been much less successful, and essentially have only been used in niche markets in a few countries (Franzinger, 2004). Some Microbial Control Agents (MBCAs) interact with plants by inducing resistance or priming plants without any direct interaction with the targeted pathogen. Other MBCAs act via nutrient competition or other mechanisms modulating the growth conditions for the pathogen (Köhl et al., 2019). Entomopathogens are either mass-produced in vitro (bacteria, fungi, and nematodes) or in vivo (nematodes and viruses) (Franzinger, 2004). Understanding the mode of action, ecological adaptations, host range, and dynamics of pathogen-arthropod-plant interactions is essential for successfully utilizing entomopathogen-based biopesticides for pest management in agriculture, horticulture, orchard, landscape, turf grass, and urban environments.

These entomopathogens are very advantageous for microbial control as their effects are specific to targeted organisms. They do not leave any toxic residues and have little or no direct impact upon parasitoids, predators, plants, vertebrates. This method also eliminates any chances of secondary pest outbreak. These pathogens are preferred by many users as it is easy to use.

3.2.3.1 Bacteria

Bacteria are relatively simple unicellular microorganisms that lack internal organelles such as a nucleus and mitochondria, and reproduce by binary fission. A wide variety of bacteria are capable of causing diseases in insects, but, as noted above, those that have received the most study are spore-forming bacilli (family Bacillaceae), especially *Bacillus thuringiensis* (Bt). Many subspecies of Bt are used as bacterial insecticides and as a source of genes for insecticidal proteins used in recombinant bacteria and Bt crops. Other bacteria that have been developed as insecticides are *B. sphaericus*, *Paenibacillus popilliae*, and *Serratia entomophila* (Franzinger, 2004). Table 6 shows the various bacterial BCAs with their target pests.

The mode of action exhibited by bacterial biocontrol agents predominantly depends on the ability to produce compounds that are inhibitory to bacterial and fungal plant pathogens infecting various crops or adversely affect the pathogen development in other ways (Narayanasamy, 2013). Bt, the most successful bacterial MBCAs, forms asexual reproductive cells, called spores, which enable it to survive in adverse conditions. During the process of spore formation, Bt also produces unique crystalline bodies. When eaten, Bt crystals dissolve in the intestine of susceptible insect larvae. The spores and crystals of Bt act as poisons in the target insects. The alkaline pH of their digestive tract causes the toxin to become activated. It becomes inserted into the insect's gut cell membranes forming a pore resulting in swelling, cell lysis and eventually killing the insect. Bt is therefore referred to as a stomach poison (Rao, 2011).

The most common strategy for constructing recombinant Bt strains is using a shuttle expression vector that contains replication origins for both *B. thuringiensis* and *E. coli*, a multiple cloning site, and genes for antibiotic resistance, for example to ampicillin and erythromycin for easy selection of transformants (Franzinger, 2004).

Table 6: Bacterial BCAs with their target pests.

S.N.	Bacterial BCAs	Target pests
1.	*Bacillus firmus*	Plant-parasitic nematodes
2.	*B. thuringiensis* var. *aizawai*	Larvae of lepidopteran (moth)
3.	*B. pumilus*	Rhizoctonia and Fusarium, as well as moulds, mildews, blights, and rusts
4.	*B. licheniformis*	Fungal species, especially those causing leaf spot and blight diseases
5.	*B. subtilis Amyloliquefaciens*	Rhizoctonia and Fusarium
6.	*B. subtilis Israelensis*	Mosquito larvae
7.	*B. subtilis Kurstaki*	Lepidopterous pests of tree fruits and vegetables
8.	*Pasteuria usage*	*Belonolaimus longicaudatus*
9.	*Pantoea agglomerans*	*Erwinia amylovora*
10.	*Pseudomonas chlororaphis*	Certain fungal species which infect plant roots and induce wilt diseases also cause root and stem rots
11.	*P. aureofaciens*	*Sclerotinia homoeocarpa*, *Colletotrichum graminicola*, *Pythium aphanidermatum*, *Microdochium nivale*
12.	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens* and *A. rhizogenes*

Source: (Kumar et al., 2018)

3.2.3.2 Nematodes

The use of nematodes as a biocontrol agent has been developed in the past two decades. These nematodes that are used for biological control of insect pests are called Entomopathogenic Nematodes (EPNs). The first nematode (*Steinernema carpocapsae*) used successfully in the control of an insect pest was reported 30 years ago from Australia (Rao, 2011). Among the 7 families of nematodes that are used for biological control, *Steinernematidae* and *Heterorhabditidae* with their symbiotic bacteria *Xenorhabdus* and *Photorhabdus*, respectively have shown the most promising results (Lichtfouse, 2010). These EPNs are preferred by many due to its immediate killing action of 24-48 hours, paired with their self-searching ability of hosts. A list of EPNs with their targeted pests is shown in Table 7:

Table 7: EPNs with their targeted pests

S.N.	EPNs	Pests targeted
1.	*Steinernema carpocapsae*	Artichoke plume moth, Armyworms, Banana moth, Billbug, Banana root borer, Black cutworm, Black vine weevil, Borers, Cat flea, Codling moth, Corn earworm, Corn rootworm, Cranberry girdler, Crane fly, Iris borer, Large pine weevil, Leafminers, Mole crickets, Navel orangeworm, Shore flies, Sweetpotato weevil
2.	*Heterorhabditis bacteriophora*	Banana moth, Billbug, Black vine weevil, Borers, Citrus root weevil, Corn rootworm, Diaprepes root weevil, Fungus gnats, Grape root borer, Iris borer, Sweetpotato weevil
3	*S. feltiae*	Armyworms, Banana root borer, Borers, Codling moth, Corn earworm, Fungus gnats, Leafminers, Shore flies, Sweetpotato weevil
4.	*S. riobrave*	Citrus root weevil, Corn earworm, Diaprepes root weevil, Mole crickets, Plum curculio
5.	*H. downesi*	Black vine weevil, Large pine weevil,
6.	*H. marelata*	Strawberry root weevil, Black vine weevil
7.	*H. zealandica*	Scarab grubs

Source: (Koppenhöfer & Grewal, 2005)

Mode of action

After locating the host/pest species, the third stage of nematodes i.e. free-living infective juveniles penetrate into its body cavity, usually via natural body openings (mouth, anus, spiracles) or through the intersegmental membranes of the insect cuticle (Elhers, 2012). Then, a symbiotic bacterium (*Xenorhabdus* for steinernematids, *Photorhabdus* for heterorhabditids) is released from the nematode gut, which multiplies rapidly and causes rapid insect death. The nematodes feed upon the bacteria and liquefying host, and mature into adults. While the bacteria is primarily responsible for mortality of insect hosts, the nematodes also produce a toxin that is lethal to the insects. The number of generations may be more than one within the host cadaver depending upon the available resources (Lichtfouse, 2010).

3.2.3.3 Fungus

Entomopathogenic fungi are among the first organisms to be used for the biological control of pests. 100s of genera with over 700 species of the divisions Zygomycota, Ascomycota and Deuteromycota have shown considerable implications of being used as entomopathogenic fungi. Among them, only 10 genera have been successfully used commercially as BCAs (Rao, 2011). These fungi nutritionally may be saprotrophs that colonize the rhizosphere and phyllosphere, endophytic saprotrophs, hemibiotrophic, necrotrophic of plants, entomopathogenic or mycoparasitic and some of them have adopted more than one eco-nutritional mode (Shah & Pell, 2003). Entomopathogenic fungi are preferred by various people for the biological control as it exerts many advantages over other BCSs such as (Khan et al., 2012):

- High degree of specificity for controlling pest without affecting beneficial insect predators and non-harmful parasites
- No hazardous effects on environment or the health of mammals unlike the chemical insecticide applications
- Different ways of infection which greatly reduces the chances of developing insect resistance
- High potentials for further development by biotechnological research as they contain genes for secretion of insect toxins
- High persistence in the environment which provides long-term suppression effects of entomopathogenic fungi on pest

Some of the widely used entomopathogenic fungi with their trade name and their targeted pests are shown in the Table 8 below:

Table 8: Entomopathogenic fungi with their trade name and their targeted pests

S.N.	Fungal BCAs	Target pests	Trade name
1.	*Candida oleophila O*	*B. cinerea* and *P. expansum*	
2.	*Beauveria bassiana* (White Muscardine Fungi)	House flies in chicken manure, Fire ants and other ants found indoors	Bio-Power, Boverol, Conidia, Naturalis, Mycontrol, Racer BB
3.	*Pythium oligandrum*	20 soil-borne pathogenic fungi	
4.	*Colletotrichum gloeosporioides* f.sp. *aeschynomene*	*Aeschynomene virginica*	
5.	*Chondrostereum purpureum*	Hardwood trees such as red alder, Sitka alder, speckled alder, and trembling aspen	
6.	*Paecilomyces lilacinus*	Plant-parasitic nematodes in soil	MeloCon WG
7.	*P. fumosoroseus* Apopka	Whiteflies, thrips, aphids, and spider mites	PFR-97, Pae-Sin
8.	*Alternaria destruens*	*Cuscuta* spp., known as dodder, swamp dodder, large seed dodder, small seed dodder, and field dodder	
9.	*Muscodor albus*	Root rot, damping off, and wilt disease-producing fungi and bacteria	
10.	*Aspergillus flavus*	*A. flavus* that produce aflatoxin	
11.	*Metarhizium anisopliae* (Green Muscardine Fungi)	Various ticks and beetles; root weevils, flies, gnats, thrips	Meta-Sin®, Green muscle, Pacer
12.	*Puccinia thlaspeos*	*Isatis tinctoria*	
13.	*Pseudozyma flocculosa*	Powdery mildew on roses and cucumbers	
14.	*Verticillium lecanii*	Greenhouse whitefly, thrips, aphids	Vertalec, Mycotal
15.	*Metarhizium flavoviride*	Locusts, grasshoppers	Green Muscle

Source: (Kumar et al., 2018)

The infection process of these entomopathogenic fungi begins with asexually produced fungal spores or conidia. These conidia are dispersed throughout the environment in which the insect hosts are present. After locating suitable hosts, the conidia attaches to the insect's cuticle. Infection structures such as germ tubes, appresoria and penetration pegs are formed that allows invasion of the insect body and circulatory system (haemolymph) (Sandhu et al., 2012). Enzymes activation occurs and various mechanisms are adopted by fungi to suppress the growth of the insect hosts or to kill them. These mechanisms include:

- Antibiosis- The fungi produces an inhibitory metabolite or antibiotic against the host.
- Mycoparasitism- The fungi derives some or all of its nutrients from the fungal host.
- Induced resistance- The fungi encourages induction of plant defence response against plant pathogen.
- Growth enhancement- The fungi promotes plant growth while the effects of the disease are being reduced and also through microbial hormones such as indole-acetic acid and gibberellic acid (Thambugala et al., 2020).

The use of entomopathogenous fungi in biological control can only be conceived in two ways: first, stimulating the natural endemic tendency of the pathogen by modifying the host insect's susceptibility, as manipulation of environmental factors can be attained only by the modification of agricultural practices; second, inducting epizootic fungal development by introducing a complementary artificial inoculum (Ferron, 1978).

3.2.3.4 Virus

Entomopathogenic viruses are obligate intracellular parasites having either DNA or RNA encapsulated into a protein coat known as capsid to form the virions or nucleocapsids (Kalha et al., 2014). Out of a total of 73 known virus families, more than 17 families or groups of invertebrate viruses have been recognized as pathogenic to insects, of which baculoviruses have been the most documented against different orders of insect-pest including Lepidoptera, Diptera, Hymenoptera, Orthoptera, Isoptera and Neuroptera (Peñaflor, 2019).

The Baculoviridae has two sub groups: Nuclear Polyhedrosis Virus (NPV) and Granulosis Virus (GV) whereas another virus group Reoviridae has a sub group called Cytoplasmic Polyhedrosis Virus (CPV) as presented in Table 9.

Table 9: Commonly used virus groups and their targeted hosts with the examples of virular strain

S.N.	Virus groups	Targeted hosts	Examples of virular strain
1.	Baculoviridae a) NPV b) GV	Lepidoptera, Diptera, Hymenoptera, Trichoptera, Coleoptera, Neuroptera, Crustacea, Mites	Ha-NPV (against *Helicoperva armivera*) Sl-NPV (against *Spodoptera litura*) Po-GV (against *Phoremia operculera*)
2.	Reoviridae CPV	Lepidoptera, Diptera, Hymenoptera, Crustacea	Dp-CPV (against *Dendrolimus pini*)

Source: (Payne, 1982)

Mode of action

The initial infection occurs when a susceptible host insect feeds on plants that are contaminated with the occluded form of the virus. When the Occluded Bodies (OBs) are ingested by the insect, releasing the infective particles (virions or ODV/PDV) into the midgut.

In the next step, uncoating of the ODV/PDVs takes place followed by the acquisition of another envelope comprising cytoplasmic membrane and the virus-coded glycoprotein. Such forms of the virus are known as Budded viruses (BVs).

Then, BVs are released into the haemolymph that undergo rounds of multiplication in the cells of susceptible tissues. PDVs are produced in the late phase of the infection. Finally, the occlusion body protein (polyhedrin/ granulin) crystallises to form the OBs, which are released into the environment. Viral proteases and chitinases help to disrupt the chitinous exoskeleton, resulting in disintegration and finally the death of the host insect (Kalha et al., 2014).

3.2.3.5 Protozoans

About 500 species of protozoans have been explored to have entomopathogenic effects and hence called Entomopathogenic Protozoans (EPP). These EPPs are generally host- specific and slow acting, producing chronic infections with general deliberation of the host. A list of EPPs with their targeted hosts is shown in the Table 10.

Table 10: EPPs and their targeted hosts

S.N.	EPPs	Hosts
1.	*Nosema acridophagous, N. cuneatum, N. locustae*	Grasshoppers
2.	*N. algerae*	*Anopheles albimanus, Culex tritaeniorhynchus*
3.	*N. fumifueranae*	Spruce budworm
4.	*N. heliothidis*	*Helicoverpa zea*
5.	*N. pyrausta*	*Ostrinia nubialis*
6.	*N. whitei*	*Tribolium castaneum*
7.	*N. spp.*	*Helicoverpa armigera, Spodoptera litura*
8.	*Vairimorpha necatrix*	*Agrotis ipsilon, Helicoverpa zea*

Mode of action

The protozoans produce spores that are ingested by the targeted hosts that germinate into their midgut forming sporoplasms. The sporoplasms are released into the target cells that cause infection in the host cells. The infection results in reduced feeding, vigour, fecundity, and longevity of the insect host as innundatively applied microbial control agents.

3.3 Approaches/Strategies of Biological Control of Insect pests in Nepal

A number of potential strategies are used to for the biological control of insect pests in Nepal that includes Classical Biological Control, Augmentation, and Conservation.

3.3.1 Classical Biological Control

Classical Biological Control(CBC) method refers to the introduction of natural enemies to a new place where they did not originate or do not occur naturally (Rao, 2011). Classical biocontrol methods are also known as importation as the major principle of classical control is to identify natural enemies that control a pest in its home location and import these enemies to the pests' new location. Many of the introductions do not result in establishment or if they do, the organism may not become pests. However, it is not uncommon for some of the introduced organisms to become pests, due to a lack of natural enemies to suppress their populations. In these cases, importation of natural enemies can be highly effective (Caltagirone, 1981).

Till now, more than 172 insect species have been successfully controlled by insect CBC agents worldwide. These CBC agents could be Micro-organisms (fungi, viruses) or Invertebrates (predators, parasites, or herbivorous arthropods) (Secretariat of the Convention on Biological Diversity, 2007). Some of the successful examples of classical biological control include the control of winter moth (*Operophtera brumata*) and the larch case bearer (*Coleophora laricella*) in North America, the chestnut gall wasp (*Dryocosmus kuriphilus*) in Japan, North America and Europe, Pineus aphids (*Pineus pini* and *Pineus boerneri*) in Chile, Hawaii and East Africa, and the great spruce bark beetle (*Dendroctonus micans*) in Europe and the Caucasus (Kenis et al., 2017).

The general procedure of Classical Biological Control is depicted through Figure-----.

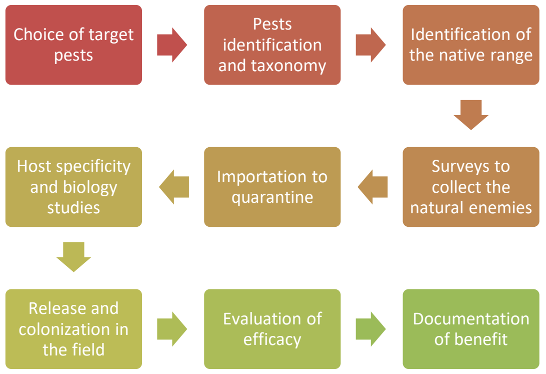

Figure 1: Procedure of classical biological control method
Source: (Hoddle & Van Driesche, 2009)

3.3.2 Augmentation

Augmentation or Augmentative Biological Control refers to the process of rearing and releasing biological control agents to effect pest suppression (Hoy, 2008). Augmentation method can be categorized into two types: Inoculation and Inundation (Toscano, 2017).

Inoculation is a preventive approach where small numbers of a natural enemy are released early in the pest cycle to prevent the pest population from building. It provides long-term control and keep pest populations from reaching excessive numbers. Ideally, the natural enemies reproduce and build in numbers along with the pest population to provide continued control throughout the season (Toscano, 2017).

Some of the examples of inoculative approach are:

- Periodic releases of the parasitoidal wasp (Encarsia formosa*)* to control greenhouse Whitefly
- Use of the predatory mite (Phytoseiulus persimilis*)* for the control of the two-spotted spider mite

Another approach is Inundation which is a remedial treatment that involves the mass release of large numbers of natural enemies to provide a quick, knockdown effect on the pest population. It also can be used to treat a pest outbreak by overwhelming a pest with the sudden introduction of large numbers of predators. The use of microbial pesticides such as Bt, *Bacillus thuringiensis*, is also a form of inundation biological control (Toscano, 2017).

Some of the examples of inundative approach as mentioned in (Hajek, 2012) are:

- Inundative release of lady beetles to control aphids
- Inundative release the predatory mite *Neoseiulus cucumeris* to control thrips
- Inundative release beneficial nematodes to control fungus gnats.
- Inundative release of fungal pathogen against locusts in Africa
- Inundative release a viral pathogen against velvet-bean caterpillars (*Anticarsia gemmatalis*) in soybeans
- Inundative release a fungal pathogen for control of the weedy stranglervine (*Morrenia odorata*) in citrus orchards.

3.3.3. Conservation

Conservation biological control is the protection and conservation of natural enemies against adverse effects of pesticides and incompatible cultural practices and improving their efficiency via providing suitable environment (El-Wakeil et al., 2017).

Some of the important strategies applied as conservation are shown in the Table 11 below:

Table 11: Important Conservation strategies used for biocontrol

S.N.	Strategies	Methods	Practices
1.	Habitat management/ manipulation	a) Increasing plant biodiversity	• Multiple cropping • Intercropping • Trap cropping • Strip harvesting • Selective retention of weeds • Conservation of wild plants at field margins
		b) Conservation tillage	• Providing food • Reducing mechanical injury • Eliminating dust • Reducing deleterious effect

		c) Artificial structures	• Tying gunny bags around citrus trees to trap and protect coccinellid predator • Building nesting boxes for predatory birds and wasps
		d) Cover crops	• Planting ground cover crops • Building suitable microclimate for predators that prey on spider, mites, aphids, etc
		e) Resistant crop varieties	• Planting resisting crop varieties
2.	Pesticide management	a) Selective pesticides	• Use of pesticides that favour natural enemy
		b) Timing of pesticides	• Choosing time of application of pesticides that allows minimum detrimental effect against natural enemies
		c) Non-intervention diseases	• Monitoring natural enemy density • Understanding their impact on pest population

3.4 Status of biological control against insects in Nepal

Nepal, a country with rich biological diversity, possesses immense potential on the control of insect pests through Bio-control Agents (BCAs). Various governmental and non-governmental bodies such as NARC, IAAS, Department of Agriculture and CIMMYT are involved in numerous projects, researches on survey and monitoring and management options. These bodies are mainly concerned on developing biocontrol methods against insects like Chickpea pod borer (*Helicoverpa armigera Hubner*), Red ant (*Dorylus orientalis Westwood*), Cutworm (*Agrotis sp*), Whiter Grub, Army worm (*Mythimna sp*), Tobacco caterpillar (*Spodoptera sp*), Fruit fly complex, Aphid (*Myzus persicae (Sulzer)*), Whitefly (*Bemisia tabaci (Gennadius)*), Citrus psylla (*Diphornia citiri Kuwayama*), and Green leafhopper (*Nephotettix virescens*).

Some of the researches going-on are shown in the Table 12 below:

Table 12: Research activities on Biological control methods going on in different institutions of Nepal

S.N.	Institution/ Organization	Research	Targeted pests
1.	NARC	a) Feeding of Green Lacewing (*Chrysoperla carnea*) to Rice moth during off-season	Aphids
		b) Research on the use of *Copidosoma Koehleri* as egg parasitoids	Potato Tuber Moth (PTM)
		c) Research on *Aphelinus mali*	Woolly apple aphids, a serious pest in Jumla
		d) Research on *Phoremia operculera* (Po-GV) strain	PTM
		e) Research on *Metarrhizium anisopliae* and *Beauvaria bassiana*	Various insects
		f) Research on the use of *Trichogramma* species as egg parasitoids	Stem borer
2.	IAAS	Trials on *Metarrhizium anisopliae* and *Beauvaria bassiana*	Fall army worm
3.	Department of Agriculture	Efficacy test of Nematodes	
4.	CIMMYT	Trials on various strain of Maize Fall army worm	

3.5 Challenges in practicing biological control methods in Nepal

Despite having a huge potential of insect pest management through biological control, various obstacles are creating difficulties to achieve the desired level of progress. Some of the challenges prevalent in this sector are:

- Lack of understanding and familiarization of new technology of biological control at farmer's level that makes them hesitant towards adopting new control methods
- Lack of knowledge and importance on integration pest management that causes maximum dependency on chemical control methods
- Inadequate data and study on crop phenology and climatic condition which are vital information while applying biological control methods
- Lack of study on the selectivity of agrochemicals to natural enemies which reduces the prospects of biological control agents
- Lack of availability and commercialization of the potential bio-products in Nepali market as these bio-products are difficult to mass-produce and integrate with other control methods.
- No provision of incentives especially to small farmers at rural area

4. CONCLUSIONS

The use of biological strategies to control insect pests started years ago. Several bacteria, fungi, viruses, protozoans, and nematodes with other higher organisms have played a significant role in integrated pest management at many places around the globe. Despite the impressive efficacy level shown by the biocontrol agents (BCAs), people have always been more inclined towards chemical pesticides. Farmers prefer the chemical control methods over biocontrol due to the quick and irresistible results delivered by chemicals, while manufacturers favour them as BCAs are difficult to process, package and distribute in markets. There aren't enough research data on crop phenology and climatic condition to assess the use of suitable BCAs. Hence, this review article is aimed at summarizing the various benefits obtained from BCAs that would encourage the farmers, researchers, and manufacturers to shift their preference towards biological control methods. This paper also discusses strategies for more efficient conservation biological control comprising classical biological control, Augmentation, and Conservation. Such biological control strategies might contribute to preserve the natural biodiversity in the agricultural environment and provide alternatives to chemical pesticides. The successful results of the incorporation of biocontrol methods in the IPM strategies at various parts of the world as mentioned in this paper are bound to revitalize the importance of biological control methods. The government with the concerned authorities should focus on developing plans and policies that promote the use of BCAs in agriculture. While these programs are likely to provide better advances for biological control in the future, there remains plenty of scope for further research into formulation and application technology to facilitate successful adoption by end-users.

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