

Review

Use of Entomopathogenic Fungi as Biopesticides to Manage Insect Pest: A Review

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Abstract: Entomopathogenic Fungi (EPF) biopesticides are more environmentally friendly and are an alternative to toxic synthetic chemicals. Due to EPF's pathogenic nature to various arthropod pests, it is thus considered a primary candidate for an integrated biological approach due to its numerous invaluable effects. However, an urgent need is to develop an environmentally safer, more sustainable, and practical approach to managing insect pests. Incorporating fungal biopesticide in an Integrated Pest Management (IPM) approach offers an opportunity to reduce the unselective and continuous use of synthetic chemicals to manage insect pests. There are limited reviews on biopesticides in developing countries concerning research questions. The review aimed to provide an understanding of the use of entomopathogenic fungal biopesticides to manage insect pests, majorly aphids in horticultural crops such as French beans." The study uses an inclusive search approach, identifying 1046 articles and reports from 2010-2022 from relevant sources like Web of Science, dimension, Google Scholar, and Google. Out of these, 85 original papers and grey literature were selected and were related to fungal biopesticide use in Kenya and aimed at improving comprehensive knowledge on the benefits and use of EPF biopesticide against insect pests, their action, and how they kill the target pest. The current review presents information on the use of EPF, *Metarhizium anisopliae*, and *Beauveria bassiana* as biopesticides that are dominantly used in Kenya and attributed to their pathogenicity, registry, accessibility, and secondary metabolites produced, thus, increased demand with more than 132,980 hectares in 2019 in Kenya under biopesticides. However, their use in biocontrol processes is still underestimated due to a lack of knowledge. Thus, this study review recommends integrating EPF with other measures for enhanced fungal biopesticide formulation, pathogenicity, and increased shelf-life.

Keywords: Microbial Biopesticides, IPM, *Metarhizium anisopliae* Aphids

Introduction

Conservation of natural enemies for insect pest through promoting sustainable agriculture is imperative for ensuring food security and maintaining the integrity of the food systems (Ochieng *et al.*, 2022; Roubos *et al.*, 2014). In this context, the detrimental effects of synthetic insecticides on the environment, human health, and natural enemies are well-documented (Alfaro-Tapia *et al.*, 2021; Kumar, 2019). To address these challenges, alternative pest management strategies such as companion crop use (Ben-Issa *et al.*, 2017; Gontijo *et al.*, 2018; Reddy, 2017;

Sarkar *et al.*, 2018) and biopesticides (Akutse *et al.*, 2020; Srinivasan *et al.*, 2019) that mimiises on the use of selective synthetic insecticides (Gebreyohans *et al.*, 2022; Roubos *et al.*, 2014). Thus, safer management approaches have garnered attention for their potential to mitigate the impact of insect pests on agricultural productivity while minimizing adverse environmental consequences and food system degradation (Ben-Issa *et al.*, 2017; Gontijo *et al.*, 2018; Akutse *et al.*, 2020). On the contrary, synthetic insecticide use is attributed to an irreversible negative impact on the environment, human health, and natural enemies (Alfaro-Tapia *et al.*, 2021; Kumar, 2019). In relation to the latter,

biopesticides containing hypocrealean fungi (e.g., *Metarhizium anisopliae*, *Beauveria bassiana*, *Akanthomyces muscarius*, *Cordyceps fumosorosea*) have been incorporated in Integrated Pest Management (IPM) for most arthropod insect pests (Akutse *et al.*, 2020; Deer *et al.*, 2021; Kumar *et al.*, 2021). Compared to synthetic chemicals, the active components of biopesticides are safe, have less residual, no postharvest interval, degrade faster, have low resistance development and impact to natural enemies, and are safer to the environment and humans (Deer *et al.*, 2021; Srinivasan *et al.*, 2019). However, sustainable integrated use of Companion Cropping and Entomopathogenic Fungi (EPF) in managing insect pests (aphids, thrips whiteflies and many more) requires careful understanding and evaluation of their impact and effects, individually and combined on pests and natural enemies, crop yield and quality of different horticultural crops in various ecological zones (Ben-Issa *et al.*, 2017; Gontijo *et al.*, 2018; Sarkar *et al.*, 2018). In Kenya, the horticultural sector, which includes producing and selling flowers, fruits, and vegetables, doubled as Kenya's most valued agriculture subsector. The total domestic production value for the horticulture sector increased from Kenya Shillings (KES) 207.5 billion in the year 2017-248.5 billion KES in 2018, which is equivalent to 19.7% increase (HCD, 2020; Kenya National Bureau of Statistic (KNBS, 2019). Horticultural exports earn the country a considerable income, with fresh vegetable exports fetching about 48% of the foreign exchange. Vegetable production directly offers food and nutritional security, increased incomes, and employment (Ng'endo *et al.*, 2018; Nordey *et al.*, 2017). French bean (*Phaseolus vulgaris* L.) is an exotic vegetable that continues to gain commercial value due to its huge demand in the export market. Kenya is Africa's second largest exporter of French beans after Morocco, contributing about 52% in value and a total export vegetable volume of 61% (Fulano *et al.*, 2021; OECD, 2021). French beans are grown mainly for their edible pods and are rich sources of nutrients such as carbohydrates, dietary fibers, proteins, vitamins, and other essential minerals (Didinger and Thompson, 2021; Myers *et al.*, 2019). Other common names for French beans are green beans, snap beans, kidney beans, haricot beans, or string beans, depending on the ecological area and location (Khondoker *et al.*, 2020). The emphasis of this study on French beans was to depict other horticultural high-value crops besides tomatoes (*Solanum lycopersicum* L) and green paper (*Capsicum annuum*) with great economic potential the economy. Despite the importance of French beans to the economy, profitability and safe crop production are hampered by several challenges. The average yield of French beans in Kenya is estimated at 5.6-8.8 tons /hectare, which falls below the world average

of 14 tons per hectare and China has an average yield of 26 tons per hectare (Mwangi *et al.*, 2019). The vegetable sector in Kenya, including French beans, has suffered a decrease in export volumes from 22% in 2016 to 16% in 2020 (HCD, 2020). The decline has been attributed to abiotic factors (drought, temperature, light, soil fertility, and relative humidity) and biotic stress, mainly diseases and pests (FAO, 2021). Other abiotic and biotic factors also contribute to the loss of French Beans' quality and yield. Bean aphids (*Aphis fabae* Scopoli) are economically considered essential insect pests, limiting the realization of maximum yields and quality of French beans.

Bean aphids are considered among the most severe pests worldwide, capable of causing 70-80% yield loss in various crops, particularly vegetables (Nordey *et al.*, 2017). The losses are either due to direct damages caused by sucking plant sap or wounding plant tissues (Boni *et al.*, 2021) or indirect damage through the transmission of pathogens to healthy plants. The honeydew secreted by aphids forms sooty mold on plant foliage and subsequently reduces leaves' photosynthetic capacity, reducing yield and quality (Wamonje *et al.*, 2020). French bean growers can use different insect pest control strategies to minimize losses, such as cultural, mechanical, and synthetic pesticides. Synthetic pesticides are most preferred in managing and controlling aphids because they are considered easy to apply (labor-saving) and accessible compared to other methods. However, irrevocable drawbacks are associated with the frequent and indiscriminate use of insecticides. These include environmental pollution (bio-magnification), threat pollinators, predators, parasitoids, and prey (Bass *et al.*, 2015; Marete *et al.*, 2021), often less effective at suppressing aphid population due to their high fecundity leading to a build-up of resistance by the pests (Mweke *et al.*, 2020). Also, the accumulation of pesticide residues in fresh and processed products (Marete *et al.*, 2020; Sharma *et al.*, 2019a). Therefore, the adverse effects of synthetic pesticides create an impetus for French bean growers to seek environmentally safe and acceptable alternative control measures against this important pest while safeguarding human health and the ecosystem.

One promising avenue is the integration of biological control agents and companion cropping practices. Conversely, natural pest regulation using biological agents such as predators, parasitoids, or entomopathogenic fungi and companion planting as an intercrop or trap crop (Colmenarez *et al.*, 2020) (Colmenarez *et al.*, 2020) is crucial and a safer alternative to synthetic pesticides. Several species of Entomopathogenic Fungus (EPF) are reported to be more effective against a broader range of agricultural pests (Mweke *et al.*, 2019), presenting an opportunity to be used to manage sucking insect pests such as aphids. Entomopathogenic fungi are beneficial groups of fungi with soil as their niche, capable of infecting insect pests

by penetrating the cuticle of their bodies and eventually killing them (Rajula *et al.*, 2021). According to Bamisile *et al.* (2021), Entomopathogenic Fungi (EPFs) are not only pathogenic to insect pests with broad host plants and induce plant pathogen antagonism mechanisms but also promote plant growth. EPFs are also sources of bioactive and secondary compounds and rhizosphere colonizers and are essential in the biotransformation of steroids and flavonoid glycosides. Farmers in other parts of the world, like Asia, North America, Europe, and South Africa, have successfully used entomopathogenic fungus products to control various pests (Khun *et al.*, 2021).

In Kenya, there is still limited empirical research on IPM approaches, such as using companion crops and EPFs to control back bean aphids. Meanwhile, according to Sarkar *et al.* (2018), companion planting is an approach to support the population of natural enemies and thus manage insect pests and biodiversification. Companion crops, on the other hand, act as either repellent or attractant of pests and can reduce pest effects on the primary crop. This has proved an effective control strategy for insect pests (aphids) in crops like collards (Gontijo *et al.*, 2018), hot peppers (Waweru *et al.*, 2021), sweet peppers (Ben-Issa *et al.*, 2017) and reduced the high usage of pesticides (Parker *et al.*, 2013). Furthermore, companion cropping is a traditional practice among most vegetable farmers and it is used to diversify income sources for the farmers due to the economic value of the additional crop. It also lowers cost and chemical residues and preserves living organisms' biodiversity within the ecosystem (Ben-Issa *et al.*, 2017). Integrating entomopathogenic fungi with companion cropping presents an opportunity to enhance their effectiveness against black bean aphids. This will ensure the safety of human health and the environment and minimize cases of development of resistance by pests to synthetic pesticides. Despite the potential of these approaches, empirical research, more understanding, and knowledge of Integrated Pest Management (IPM) strategies, particularly in the context of French bean production in Kenya, remains limited (Sarkar *et al.*, 2018). Therefore, this study aims to bridge this gap by investigating the efficacy of companion cropping and biopesticides, specifically entomopathogenic fungi, in managing black bean aphids in French beans. By providing insights into the practical application of integrated sustainable pest management strategies, particularly on microbial biopesticides, this research seeks to enhance French bean production while promoting environmental sustainability and human health concurrently with a more in-depth understanding of biopesticides use in different agro-ecology.

Methodology

This review used a monographic approach based on unpublished and published findings. An inclusive search approach was developed to identify other literature relevant to the topic of study. The search ranged from articles, journal papers, books, and book chapters to government sector and development partner reports found in different search engines such as Elsevier, Wiley Online, and Springer from 2010-2022, shown in Fig. 1 and showing the increased trend of interest in the area of microbial biopesticides. Specific search words include “biological control of aphids and their impact on the quality and shelf life of French beans.” This was to document the different integrated biological control strategies linked to entomopathogenic fungi “*Metarhizium anisopliae*” used to manage insect pests, especially aphids, and the quality and productivity of French beans in Kenya. The database was selected to retrieve the literature publications covering our objectives. The author then independently assessed the scientific articles identified in the latter database. Out of 1046 papers and reports, rigorous sorting was done based on the relevance to the subject matter, and only 89 original papers and grey literature were selected and used for this study. Articles considered appropriate were included based on title and abstract and if they did not meet all eligibility criteria, the full text was examined for further evaluation. The current trend in publications based on the search shows more attention and expansion of interest to biopesticides as sustainable alternatives to synthetic insecticides. An aspiration by scientists and high demand by consumers for safe produce, moreso heightened by the public and government authorities' quest for a sustainable, safe, and integrated approach to manage most agricultural pests is attributed to the sharp trend of scientific publications in different databases and the current interest in this topic.

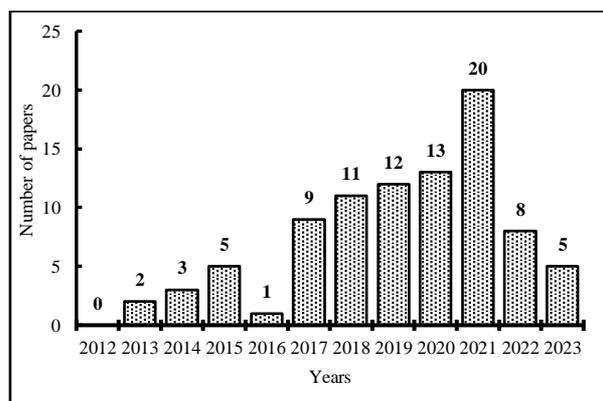


Fig. 1: Shows the total number of papers used in this current review study per year (2012-2022)

Classification of Pesticides According to Their Sources

Pesticides are classified into chemical and biological pesticides based on their derived source (Ayilara *et al.*, 2023). Unlike chemical pesticides made from organic and inorganic compounds, they are considered very effective and rapid in controlling insect pests. The fast knock characteristic of chemical pesticides is attributed to high solubility, easy absorbability by pests, and adhering to plants' surface, enhancing their activity and durability in the environment (Khun *et al.*, 2021). As a result, chemical pesticides significantly contribute to agricultural productivity through timely management of pests. However, in 2019, according to the World Health Organization (WHO) and the Food and Agricultural Organisation (WHO), the continuous and indiscriminate usage of synthetic pesticides poses a grave threat to humans and the environment. First documented in the book "Silent Springs" by Rachel Carson, it led to a swift need for sustainable, safe alternatives.

For this reason, to address the increased concern about poisoning, carcinogenic illness, and loss in biodiversity associated with the use of synthetic pesticides thus, the production of new pesticides became necessary. Therefore, biopesticides were/are considered potential alternatives since they are safe for humans and the environment. Biological pesticides are derived from microorganisms (fungi, bacteria, viruses, and protozoa) and plants (phenols, alkaloids, and terpenes). As a result, biopesticides are considered eco-friendly, cheap, and sustainable, with no residues, and not associated with greenhouse gases. They can also be used as biological control agents. Unlike most synthetic pesticides with neurotoxic modes of action, most microbial pesticides are host-specific, exhibiting anti-feeding, desiccation, suffocation, and distrust mating of target pests. Besides, biopesticides have biological interactions with plants and natural enemies thus have shown great potential against a wide range of arthropods (sucking insects, borer, defoliators, miners, etc.) on plants' vegetative parts in the different ecosystems (Ayilara *et al.*, 2023; Irsad *et al.*, 2023; Kumar *et al.*, 2021; Marwal *et al.*, 2022).

Advantages of Microbial Biopesticides

Besides the high cost of production (screening, developing, and regulatory clearance; short shelf life due to their sensitivity to sunlight, temperature, and humidity; high doses for open field conditions; and need for technical knowledge. Because of their nature and characteristics, biopesticides degrade quickly, have less bioaccumulation in the environment, and prevent or stop soil and water pollutants. Table 1 shows the merits and demerits of microbial biopesticides (Ayilara *et al.*, 2023;

Daraban *et al.*, 2023; Irsad *et al.*, 2023; Kumar *et al.*, 2021). Microbial biopesticides do offer a potential alternative to synthetic methods due to their wide host range (more than 200 species in different orders), reduced resistance, no residues, zero pre-harvest interval, safe to humans, and less harmful to the environment benefits (Mweke *et al.*, 2018).

Insect Pest Biology, Life Cycle, and Their Impact

Aphis fabae Scopoli 1763 (*Hemiptera: Aphididae*) is a highly polyphagous insect pest and lives in clusters on plant tender parts like stems, petioles, flowers, and pods (KBS, 2021). Aphids show polyphenism behavior (ability to have wings or not). They reproduce either by asexual or sexual reproduction, depending on the ecosystem conditions (Kumar, 2019; Mehrparvar *et al.*, 2013). The adult female can hatch 40-100 live wingless nymphs in her lifetime. Adult aphids land on a suitable host plant and deposit numerous live nymphs on tender plant tissues. The nymphs start to feed on plant sap and mold four times, increase in size and become adults in 7-10 days under favorable conditions. The cycle starts when mature adults deposit eggs or nymphs on the host plant, depending on the conditions, and live for about four weeks before they die (Barbercheck, 2014). Due to aphids' faster and shorter reproductive rate, they can build up resistance to commonly applied synthetic pesticides with a short duration (Mehrparvar *et al.*, 2013). Winged aphids are formed in search of new hosts due to limited resources, predation, overcrowded colonies, and the release of stress alarm pheromones. Aphids also possess a more developed sensory system for finding new habitats, are resistant to starvation, and are sensitive to environmental cues (Mehrparvar *et al.*, 2013). Most tender horticultural crops, like French beans, are preferred by plant sap-sucking and viral transmission by black bean aphids, resulting in significant economic damage and immature plant death (Valenzuela and Hoffmann, 2015). In the sap-sucking process, black bean aphids reduce and divert valuable nutrients for plant growth for their use. In response to the injuries caused by aphids, a plant often activates swift responses like oxidative burst, self-protective proteins, build-up of secondary metabolites, yellowing, leaf rolling, necrosis, and galling (Kaur *et al.*, 2017; Singh and Singh, 2021). Sooty mold deposition on plants' morphological structures, such as leaves and stems, due to the high aphids incidence and colonies increase fecal secretion, reducing plant photosynthesis and respiration rate (Singh and Singh, 2021). Therefore, timely control and management of aphids could result in double yields and improve the quality of French beans, thus increasing their penetration rate to the international market.

Table 1: Graphical illustration of microbial biopesticides (advantages and disadvantages)

Microbial biopesticides	
Advantage	Disadvantage
Host-specific (in their mode of action)	High doses required
Environmentally friendly	Slow in action compared to synthetic
Sustainable pest management approach	Limited or short self-life
From different species, thus wide range	of Application methods with multiple time
Not associated with greenhouse gases	Limited due to their high availability
Less expensive compared to synthetic	Multiple application rates are needed
No likelihood of resistant development	Effective over time than synthetic
No bioaccumulation to the environment	Stringent regulation on their use
Secondary infection/ self-sustainability	Easily degraded (U.V light and heat)
Compatible with IPM methods	
A sustainable and preventive approach	
No pre-harvest interval	

Bean Aphid Damage Effects

For most tender horticultural crops like French beans, plant sap-sucking, and viral transmission due to black bean aphids result in significant economic damage and immature plant death (Valenzuela and Hoffmann, 2015). In the sap-sucking process, black bean aphids reduce and divert valuable nutrients for plant growth for their use. In response to the injuries caused by aphids, a plant often activates swift responses like oxidative burst, self-protective proteins, build-up of secondary metabolites, yellowing, leaf rolling, necrosis, and galling (Kaur *et al.*, 2017; Singh and Singh, 2021). Sooty mold deposition on plants' morphological structures, such as leaves and stems, due to the high aphids incidence and colonies increase fecal secretion, reducing plant photosynthesis and respiration rate (Singh and Singh, 2021). Therefore, control and management of aphids would potentially double the yields and quality of horticultural crops.

Management Approach

Studies have shown that chemical pesticides have been developed to increase yield and income among farmers in Kenya, with systemic and contact pesticides like alpha-cypermethrin, imidacloprid, dimethoate, deltamethrin, lambda-cyhalothrin, Beta-cyfluthrin, and Abamectin being effective against aphids. However, increased application frequency threatens human health, environmental pollution, residual accumulation, and pest resistance. A study by Omwenga *et al.* (2021) found that tomatoes (22%) had the highest residues of different pesticides above the Maximum Residue Limit (MRL) accepted on produce, followed by French beans (21%), Kale and Spinach respectively. Compared to biopesticides, synthetic chemicals reduce vegetable quality and pose severe threats to consumer health and the environment. Omwenga *et al.* (2021) reported that generally, in Kenya, the amounts of synthetic pesticides applied in vegetable crops are thrice more than in cereal crops. Most vegetable farmers prefer using synthetic pesticides to control different agricultural pests because of

their quick effectiveness, affordability, and accessibility to enhanced productivity to fetch more income (Bass *et al.*, 2015). However, this has encouraged indiscriminate and increased application frequency that poses threats to human health, environmental pollution, residual accumulation, and development of resistance by the pests (Alfaro-Tapia *et al.*, 2021; Marete *et al.*, 2021; Sharma *et al.*, 2019b). Repetitive pesticide application poses environmental and human health threats. Studies show that workers in horticulture face health complications from synthetic pesticides (Tsimbiri *et al.*, 2015). According to a study report by Marete *et al.* (2021), about 350,000 cases of pesticide poisoning occur annually in Kenya, with 26% of farmers experiencing health effects in Meru county. Thus, increased health problems associated with synthetic pesticide use are attributed to the continuous lack of alternative and safer management strategies that are more effective for this aphid population than synthetic pesticides (Kim *et al.*, 2020). As a result, several drawbacks associated with the indiscriminate use of synthetic pesticides lead to excess residues, pollution, and harm to non-target organisms. Kumar and Omkar (2018) observed that repeated use leads to pest resurgence, resistance build-up, pollination, secondary pests, and human health hazards. Biopesticides containing *Metarhizium anisopliae* are recommended as an environmentally safe alternative for aphid management, showing no side effects, reduced resistance and zero pre-harvest intervals can effectively suppress aphid pest population (Boni *et al.*, 2021; Mweke *et al.*, 2018; Reingold *et al.*, 2021). The later research confirms the effectiveness of biopesticides in reducing the aphid population relative to synthetic pesticides; however, field conditions directly affect the performance of biopesticides (ultra-violet light, heat, drought, humidity, etc.).

On the other hand, physical management approaches, such as warm water treatment, flooding, bagging, roughing, hand picking, and trapping, have been used to control various insect pests (Kumar and Omkar, 2018) Nawaz *et al.* (2016). However, these methods can be costly for smallholder farmers, requiring labor and time, making them less effective and practical. For example, agronet covers by Gogo *et al.* (2014) can reduce Silverleaf whitefly (*Bemisia tabaci*) populations and black bean aphids in French beans. Overall, a more cost-effective approach is needed for effective pest management. Alternatively, cultural methods like crop rotation, intercropping, and field sanitation can increase crop productivity. Intercropping kale with culinary herbs reduces the aphid population and losses. However, intercropping alone is insufficient for pest management. Border crops like sorghum, maize, and sunflower reduce aphid-transmitted viral diseases Hendges *et al.* (2018). Waweru *et al.* (2021) found decreased aphid-transmitted viral diseases in hot peppers when border crops like

sorghum, maize, and sunflower were used, but not on the main crop. Mixed cropping (Tesemma *et al.*, 2010) is standard in East Africa, particularly in Kenya, to diversify income sources, replenish the soil, manage pests, and spread risk. Ben-Issa *et al.* (2017); Sarkar *et al.* (2018); Reddy (2017) study report the use of intercrops, such as sunflower and nasturtium, as traps to attract pests and lure them away from the main crop. Insecticides applied to trap crops reduce application rate, chemical exposure, and costs, enhancing product quality and shelf life. Mweke *et al.* (2020) study research showed that entomopathogenic fungus can control *Aphis craccivora* Koch's population without affecting natural enemies. Sunflowers have been used as trap crops to manage insect pest populations (Sarkar *et al.*, 2018; Shelton and Badenes-Perez, 2006). Other authors (Ceolin Bortolotto *et al.*, 2015; Khan *et al.*, 2017; Parker *et al.*, 2013; Sharma *et al.*, 2019c) have also documented the successful use of Wheat (*Triticum aestivum*. L) as a companion crop for aphids to manage their populations. Field bean intercropping with wheat and barley reduced black bean aphids and infested plants, increasing profits by 42 and 70%, respectively, according to a study by Hansen *et al.* (2008). Companion cropping is essential for pest management, allowing for self-conservation, pest regulation, and ecosystem stability (Amala and Shivalingaswamy, 2018; Ben-Issa *et al.*, 2017). It also provides shelter and food for beneficial pollinators and predators (Mwani *et al.*, 2021). Research shows that companion crop and intercropping approaches are effective; incorporating other IPM strategies like biopesticide and multiple trap crops could improve their effectiveness. However, cultural control approaches are less effective and have drawbacks, necessitating research on integrated pest management approaches and an in-depth understanding of synchronizing the approach with pest infestation.

Biopesticides as Biological Control Agents for Insect Pests in Kenya

Biological Control. Biological control dates back to ancient Egyptians, around 4,000 years ago (Kwenti, 2017). After World War II, there was a significant decline in the use of biological control due to the innovation of synthetic pesticides to increase productivity (Payton Miller and Rebek, 2018; Teresa *et al.*, 2019). Between the 1960s and 70s, pesticide resistance by pests surfaced. The period has led to the conceptualization of the Integrated Pest Management (IPM) approach to tackle the drawback of synthetic pesticides to non-targeted organisms and environmental pollution documented by Rachael Carson in 1962 (Bass *et al.*, 2015; Boni *et al.*, 2021; Carson *et al.*, 1994). Moreover, it aims to reduce residual accumulation, resistance, or cross-resistance of pests and pest resurgence

due to synthetic pesticides (Matere, 2020). Biological control strategies like predators, parasitoids, and EPF have been used commercially to manage the aphid population (Sharma *et al.*, 2019c). *Metarhizium anisopliae* (Metschn) Sorokin (*Hypocreales: Clavicipitaceae*) is an essential entomopathogenic fungus with a broad host range (Srinivasan *et al.*, 2019; Villamizar *et al.*, 2021). It is a broad pathogenic fungus to more than 200 species of insects belonging to different orders like *Coleoptera*, *Lepidoptera*, *Orthoptera*, *Hemiptera*, and *Thysanoptera* (Akutse *et al.*, 2020; Boni *et al.*, 2021; Iwanicki *et al.*, 2019). *Metarhizium anisopliae* is also known as green muscardine fungus. Different *Metarhizium anisopliae* formulation developed in Kenya by Realipm and International Centre of Insect Physiology and Ecology (ICIPE) as biopesticides account for the increased area usage from 43,290 hectares in 2015 to more than 132,980 hectares in 2019 as shown in (Akutse *et al.*, 2020) Fig. 2.

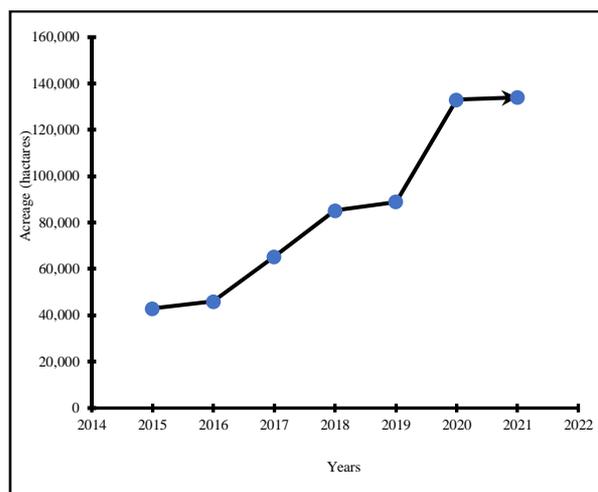


Fig. 2: Total acreage of biopeptides used in Kenya per year between 2014 and 2021

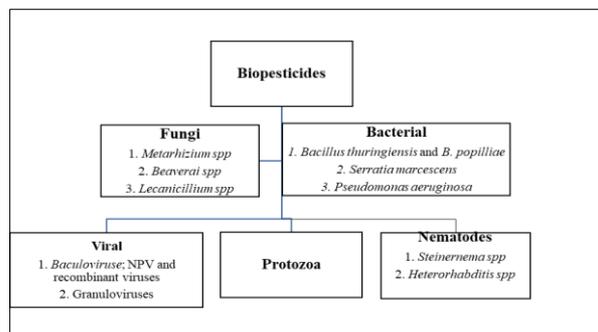


Fig. 3: Graphic illustrating the categories of entomopathogenic pathogen and their examples used to derive biopesticides to manage insect pests in Kenya

More than 37,600 farmers use *Metarhizium* spp products in sub-Saharan Africa, as reported by ICIPE (2019). In Kenya, commercialized EPFs include *Lecanicillium lecanii*, *Metarhizium anisopliae*, *Beauveria bassiana*, and *Isaria* spp (Mweke *et al.*, 2019). Different products containing *Metarhizium anisopliae*, in particular, are available in different brand names like Metarril WP (Koppert Biological System), Mazoa Supreme (Real IPM), and ICIPE62 (Srinivasan *et al.*, 2019). Numerous groups of pathogens that cause disease to insect pests include entomophytes (*Trichoderma*, *Hypocrea*, *Bionecteria*, *Clonostachys*), entomopathogenic fungi (*Beauveria*, *Metarhizium*, *Verticillium*, *Isaria*), entomopathogenic bacteria (*Bacillus thuringiensis*, *Serratia marcescens*), entomopathogenic nematodes (*Heterorhabditis* and *Steinernema*) and Baculoviruses (*Spodoptera exigus* NP and *Spodoptera littoralis* NPV) shown in Fig. 3. In addition, the different fungal biopesticides registered under Kenya's Pests Control Products Board (PCPB), their active ingredient, agents, target pests and crops are available on the market, as shown in Table 2. EPFs have a broad host range but are more specific. Insect pests such

as aphids have also been shown to be susceptible to infections caused by fungi and capable of under natural conditions to regulate their populations. There is a need to introduce more efficient strains of entomopathogenic fungi than those already occurring in an area. The EPFs infest pests through physical penetration or enzymatic degradation of the cuticle, then secrete toxins that kill; hence, the pest is less likely to build resistance and cause adverse effects than the chemical. Thus, it presents an alternative pest management approach for bean aphids and minimizes the use of synthetic pesticides. The EPFs are rendered safe for humans, less harmful to the environment, relatively host-specific, reduced resistance build-up, low residues, and zero pre-harvest intervals requirements compared to chemical pesticides (Akutse *et al.*, 2020; Kumar *et al.*, 2021; Mweke *et al.*, 2018). The study suggests strategies for integrating microbial biopesticides into an integrated approach, such as pest pressure assessment, thorough monitoring, selection of compatible microbial biopesticides, application techniques, adoption of compatible farming technologies, monitoring, adaptive management, and education.

Table 2: Different fungal-based biopesticides registered in Kenya, target pest and their active ingredient; sourced Pest Control Products Board (PCPB) by 2022

Trade name	Registration number	Active ingredient	Source/ manufacturer	Target pest	Crop
Mazao Achieve	PCPB(CR)1229	<i>Metarhizium anisopliae</i> ICIPE 78 1×10^{11} cfu/mL	ICIPE	Spider mites Fall armyworm	Roses maize
Biomysis Mean 1.15% W.P. Wettable Powder	PCPB(CR)2207	<i>Metarhizium anisopliae</i> strain (Meichikoff.) <i>Sorokin</i> (NCIM-1311) 1×10^8 cfu/mL	Varsha bioscience and technology India Private Ltd, India	Thrips and caterpillars thrips, caterpillars and mealybugs thrips	French beans Roses Chives
Bio Magic 1.5 LF	PCPB(CR)1624	<i>Metarhizium anisopliae</i> 1.0×10^9 cfu/mL	T. Stanes and Company Ltd, India	Aphids and thrips Aphids, whiteflies and thrips	Roses French beans
Real metarhizium Od	PCPB(CR)1638	<i>Metarhizium anisopliae</i> ICIPE 69 1.0×10^9 cfu/mL	ICIPE	Mealybugs	Roses
Bio nematons liquid spores and mycelial fragments	PCPB(CR)1308	<i>Paecilomyces lilacinus</i> (1.5%) 1×10^8 cfu/mL	T. Stanes and Company Ltd, India	Nematodes (root-knot, cyst, burrowing nematodes) Cyst nematodes	Roses, french beans and tomatoes potatoes
Biocatch 1.15wp wettable powder	PCPB(CR)103	<i>Verticillium</i> (<i>Lecanicillium</i>) <i>Lecanii</i>	T. Stanes and Company, India	Aphids Aphids and whiteflies	Roses and French beans tomatoes
Bio Catch 1.5 LF aqueous solution	PCPB(CR)1443	<i>Verticillium-Lecanicillium Lecanii</i> 1×10^9 cfu/mL	T. Stanes and Company, India	Whiteflies, aphids and thrips aphids and thrips	Roses and french beans tomatoes
Lecatech WP wettable powder	PCPB(CR)1144	<i>Lecanicillium lecanii</i> 1×10^{10} cfu/g	Dudutech integrated pest management limited	Thrips and whiteflies whiteflies whiteflies	French beans Roses
Mycotal WP	PCPB(CR)1358	<i>Lecanicillium muscarium</i> strain Ve6 1×10^{10} spores/gram		Whiteflies	Greenhouse roses
Bio-Power 1.15wp wettable powder	PCPB(CR)0766	<i>Beauveria bassiana</i> strain GHA 1.15% w/w		Aphids and diamond black moth	Cabbages
Biopower 1.5 liquid	PCPB(CR)1364	<i>Beauveria bassiana</i> 1.0×10^8 cfu/mL		Aphids, bollworms, caterpillars, cutworms and thrips All above and DBM	French beans and tomatoes cabbages
Beauvitech Wp wettable powder	PCPB(CR)1092	<i>Beauveria bassiana</i>	Dudutech integrated pest management limited	Thrips and whiteflies thrips	French beans Roses
Botanigard ES emulsifiable suspension	PCPB(CR)0585	<i>Beauveria bassiana</i> strain GHA 11.3% w/w	Laverlam international corporation USA	Thrips, aphids and whiteflies	French beans, Snow peas and Roses

Table 2: Continue

Boveril Wp wettable powder	PCPB(CR)2159	<i>Beauveria bassiana</i> 1×10 ⁸ spores/gram	Koppert Brazil	Whiteflies	Roses
Diptera DF dry flowable	PCPB(CR)0919	Myrothecium verrucaria 90%			Ornamentals
Aflasafe KE01	PCPB(CR)1419	Atoxigenic		Aspergillus flavus	Maize

Entomopathogenic Fungi Description and Their Mode of Action

One of the most successful biological control approaches for controlling and managing insect pests is the use of the EPF, an alternative to synthetic chemicals that employ naturally occurring microorganisms to impede the activities of insect pests and suppress their population (Sharma and Sharma, 2021). Entomopathogenic fungi that cause fungal infections are diverse organisms with various ecological functions. For instance, soil-dwelling genera *Metarhizium* and *Beauveria* regulate natural arthropods' natural populations and establish intricate connections with plants such as plant roots, stems, and leaves endophytes (Jaber and Enkerli, 2017). Research has demonstrated that *Metarhizium robertsii* and *Beauveria bassiana*, but not *Lecanicillium lecani*, supply plants with nitrogen that is absorbed as they parasitize insects (Behie and Bidochka, 2014; Litwin *et al.*, 2020) promoting plant growth such as plant height (Bamisile *et al.*, 2018; Mantzoukas *et al.*, 2022). *Beauveria bassiana* is an endophyte that occurs in about 25 plant types and suppresses plant diseases caused by fungi and pests (Sui *et al.*, 2023). In addition to colonizing plant roots, fungal endophytes and epiphyte also invades leaves and shoots, increasing plant resistance to insects (Litwin *et al.*, 2020; Ramakuwela *et al.*, 2020; Sui *et al.*, 2023). Thus, it effectively shields plants from microbial pathogens by reducing disease-causing agents, boosting plant defense responses, and protecting them. *Lecanicillium* L can also grow on the surface of leaves, enhance plant-pathogen interaction by producing antimicrobial chemicals, and trigger plant responses to root pests. Besides, in Kenya, the number of strains for microbial biopesticides derived from fungi accounted for 65%, followed by those derived from bacteria at 33% and least from viruses, shown in Fig. 4 at the ICIPE strain bank.

Meanwhile, the uniqueness of pathogenic to other pathogenic is attributed to their mode of action, which is contact-based, thus influencing their performance efficiency. How do pathogenic fungi kill their host? The pathogenic fungus can infect the host by directly penetrating the insect cuticle through combined physical pressure and cuticle-degrading enzymes (Villamizar *et al.*, 2021) in contact with the suitable host cuticle using hydrophobic interaction and adhesion. Fungal conidia or asexual spores germinate, conidia, and develop infective peg. The developed infective peg penetrates the host cuticle. The use of mechanical pressure from the

appressorium action of cuticle-degrading enzymes like trypsin, metalloproteases, and aminopeptidases is detailed in Fig. 5. The fungus hyphae penetrate the host hemocoel to obtain nutrients, releasing insecticidal toxin cyclic peptide substances called destruxins that affect host immunity. This eventually kills the host and infective conidia re-emerge from the mummified host body to infect a suitable healthy host (Boni *et al.*, 2021; Brunner-Mendoza *et al.*, 2019). Reingold *et al.* (2021) study found infective green spores on host cadavers capable of infesting new healthy pest populations and causing death. This makes the use of microbial insecticides more effective for sucking insects, in particular aphids, because of the need for only contact with the suitable target host to cause an effect in the form of a disease that suppresses the host population.

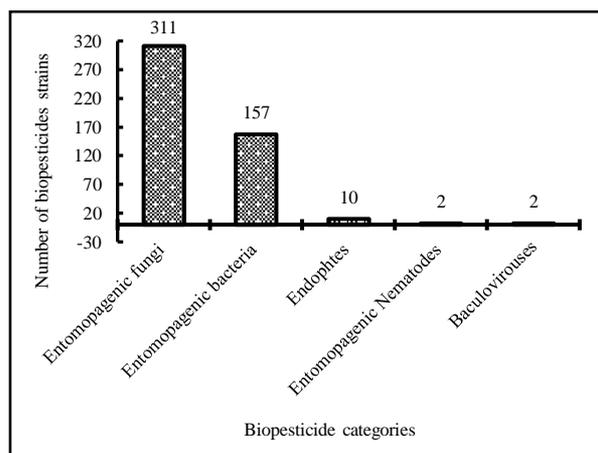


Fig. 4: Strains of arthropod pathogen for biopesticides advancement at the International Centre of Insect Physiology and Ecology (ICIPE) at germplasm bank

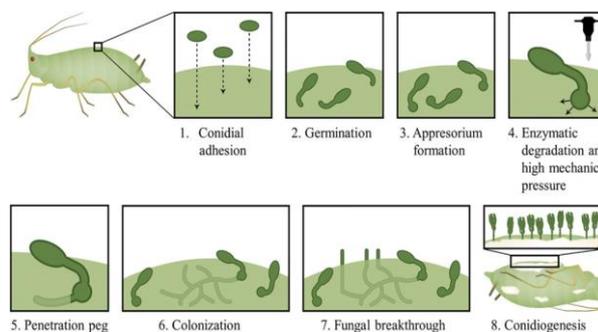


Fig. 5: Mode of action of *Metarhizium anisopliae* as a pathogenic fungus in steps (Reingold *et al.*, 2021)

Effect of Entomopathogenic Fungi *Metarhizium anisopliae* on Aphids

Use Entomopathogenic Fungi (EPF) containing *Metarhizium anisopliae* as the active ingredient research by Bayissa *et al.* (2017) greenhouse trial of kale and okra found that a product containing *Metarhizium anisopliae* as the active ingredient accounted for about 73-98% mortality rate to cabbage aphids, cotton aphids and turnip aphids seven days after inoculation. Mweke *et al.* (2018) reported that *Metarhizium anisopliae*, under laboratory tests, was less pathogenic against the aphid predator *Cheilomenes lunata*. However, they had high conidial production, responsible for a 34.5-90% mortality rate in the *Aphis craccivora* population (Mweke *et al.*, 2018). Yun *et al.* (2017) observed that entomopathogenic fungal isolates containing *Metarhizium anisopliae* and *Beauveria bassiana* successfully control and manage the green peach aphid. Murerwa *et al.* (2015) observed higher virulence of *Metarhizium anisopliae* compared to *Beauveria bassiana* against *Rhopalosiphum padi* and *Metopolophium dirhodum* aphids. Further, *Metarhizium anisopliae* is recommended because of its ease of multiplying and lower contamination rates from opportunist microorganisms compared to other fungal isolates. Mkiga *et al.* (2021) observed that combining EPF *Metarhizium anisopliae* ICIPE 69 with sex hormone effectively suppressed the False Codling Moth (FCM) population in an orange orchard and increased the marketability yield. Sajid *et al.* (2017) observed 83.23% effectiveness of biopesticides containing *Metarhizium anisopliae* in the in vitro control of mustard aphids fed on kale leaves compared to *Beauveria bassiana* (78.33%) and *Bacillus thuringiensis* (73%), respectively. According to Kim *et al.* (2020); Srinivasan *et al.* (2019), the use of microbial pesticides, in particular, the entomopathogenic fungi, is safe, cheaper, and with a broad host range compared to the synthetic pesticides with increased negative folds on the environment and humans. Fungal production costs are lower than synthetic pesticides because they naturally colonize the soils, plant roots, plant parts, and insects as rhizosphere and endophyte colonizers (Mweke *et al.*, 2020).

Conclusion

Based on the study, sustainable agriculture that relies on integrated pest management approaches is being promoted as an alternative to synthetic approaches, particularly in developing countries. Microbial, fungal biopesticides *Metarhizium anisopliae* and *Beauveria bassiana* biopesticides are the most widely used in Kenya, followed by *Lecanicillium lecanii*, bacterial (*Bacillus thuringiensis*) and the least derived from viral pathogens (*baculovirus* and *granulovirus*) registered in Kenya for different agro-ecological regions. Microbial biopesticides

accounted for more than 132,980 hectares in Kenya in 2019, with over 37,600 farmers in sub-Saharan Africa.

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Author's Contributions

Anthony Emaru: Conceptualization, literature review, analysis, drafted and compiled and reported. Data interpretation and visualization.

Jane Gesimba Nyaanga: Reviewed, conceptualized, guided the designed, and built up the document direction.

Saidi Mwanarusi: Contributed to writing and editing, provided expertise and insights, conducted literature reviewed, and offered critical feedback.

Ethics

The authors to consent to address any ethical issues that may arise after the publication of this manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Lists of Abbreviations

- EPF - Entomopathogenic Fungi.
FAO - Food and Agricultural Organisation.
HCD - Horticultural Crops Directorate.
IPM - Integrated pest management.
MRLs - Maximum residual limits.
WHO - World Health Organisation.