Herbal Extracts as Bioinsecticides for sustainable Agriculture

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There is no doubt that the need to improve agricultural productivity and enhance its sustainability is one of the most significant challenges facing humanity. In order to feed the dramatically growing global population, food production must increase by 70 percent. The damage caused by insect pests is one of the most important factors leading to the reduced production of major crop plant species. With this projection, combined with increasing demand for sustainable agricultural practices, research is required in order to produce more toxicologically and environmentally benign pesticides to sustain future agricultural production and global food security. Synthetic chemicals are generally used to control insect pests, which cause harmful impacts on the environment and non-target living systems including human beings. For these reasons, biopesticides are gaining increasing importance as they are alternatives to chemical pesticides and are a major component of many pest control programs worldwide. The most important biopesticides on the market in commercial terms are microbial pesticides, pyrethrum, rotenone, neem oil and various essential oils. This chapter details the benefits of biopesticides, offering a full spectrum and review of the process to identify, evaluate, and develop new biopesticides. It describes the range of oil and plant extracts that may be used in the biological control of insects, and their modes of action. Finally, the chapter describes new opportunities for developing biopesticides.

Keywords: Pest management; Pesticide; Biopesticides; Extracts; Microbial; Sustainable development

1. Introduction

Agriculture is an important resource to sustain global economical, environmental and social system. There are estimated to be around 67 000 different crop pest species (including insect, plant pathogen and weed pests) and together destroy about 30-40% of world's food production [1]. Pesticides are often the only practical way to control pests before they harm us or our crops from time immemorial. The term pesticide is defined by FAO (1990) as chemicals designed to combat the attacks of various pests and vectors on agricultural crops, domestic animals and human beings, and this broad term include terms insecticides, herbicides, fungicides, rodenticides, wood preservatives, garden chemicals and household disinfectants [2]. For centuries humans have used natural or synthetic preparations as pesticide, and their use has become more pronounced with time due to increased population paralleled with decreasing soil fertility, however their use does not always decrease crop losses. For example, despite the more than 10-fold increase in insecticide use in the United States from 1945 to 2000, total crop losses from insect damage have nearly doubled from 7 to 13% [3]. Agriculture will increasingly be expected to provide not only food for a world population continuously growing, but also crops for their conversion into renewable fuels and chemical feedstocks. For these reasons, there is no doubt that this problem impacts the worldwide economy, with the increasing pressure to improve agricultural productivity in a sustainable manner. For this reason, the global challenge is to secure high and quality yields and to make agricultural production environmentally compatible.

In the 20th century synthetic insecticides have replaced natural ones, and appeared great numbers of effective compounds as organophosphate insecticides (1960s), carbamates (1970s), pyrethroids (1980s) and herbicides and fungicides (1970s–1980s). Current insecticides act primarily on four nerve targets, which are present in animals but not plants: acetylcholinesterase (organophosphorus and methylcarbamates), voltage-gated sodium channels across the nerve membrane (pyrethoids and DDT), acetylcholine receptor (neonicotinoids), γ-aminobutyric acid receptor and glutamate-receptor chloride channels (polychlorocycloalkanes, avermectins, and spinosyns) [4-6]. In the case of bioinsecticides, their mechanisms of action are extremely variable and it is not always understood [7].

However, the intensive and indiscriminate use of pesticides has resulted in some detrimental consequences on environment such as groundwater pollution, river eutrophication, toxicity to non-target organisms, and the selection of strains not only resistant to the selecting compounds but also cross-resistant to other pesticides acting at the same target. Many of the pesticides currently being used have the tendency to survive in plants for a long time. They also enter the food chain and are found in meat and dairy products and remain as residue in the soil and ecosystem for long periods of time [8-9]. It also has negative impact on health with human poisonings and their related illnesses [10]. Keeping all these facts in mind, together with the new regulatory requirements and the demand of the public for safer foods, should stimulate the development of eco-friendly alternatives to chemical pesticides to generate higher quality and greater quantity of agricultural products in a sustainable mode. These new products must be effective, biodegradable, environmentally safe and innocuous.

1.1 Biopesticides

In 2003 the Advisory Committee on Pesticides (ACP) investigated the prospects for developing alternatives to pesticides in the UK. The report recognised that while there are a number of difficulties in developing alternatives to pesticides, many of these alternative methods are viewed favourably by large sections of the general public, and therefore this presents a strong argument for their development and wide usage. However, from the farmers' and retailers' point of view their benefits are less certain. Their efficacy is often lower than conventional pesticides, and they are more variable in effect than conventional pesticide sprays (Advisory Committee on Pesticides, 2003) [11].

In this context, biopesticides are a particular group of crop protection tools used in an integrated pest management. In very general terms, according to the United States Environmental Protection Agency, it could be defined as living organisms and/or their natural products that prevent, control or suppress pest populations by nontoxic mechanisms, which pose less threat to the environment and to human health.

Some common benefits and disadvantages of biopesticides in comparison with conventional pesticides are shown in the table below (Table 1) [12]. In sum, biopesticides tend to be less toxic, more quickly biodegradable, and more targeted to the specific pest. Biopesticides are often designed to control a pest population to a manageable level rather than completely eradicate a target pest. These technical differences translate into benefits to humans and ecosystems including increased food safety, worker safety, and reduced concerns for development of pest resistance to existing control tools.

Table 1 Pros and cons of biopesticide active ingredients in comparison with conventional pesticides.

Benefits	Disadvantages
Less toxic and less environmental load Reduces their impact on beneficial and non target organisms.	*Toxicity* Several botanical insecticides are more toxic to humans and fish than a number of synthetically derived insecticides.
Rapid biodegradation Rapid degradation under environmental conditions such as sunlight, humidity, and rainfall.	*Limited field persistence and a short shelf life* More frequent applications may be necessary. Have relatively critical application times.
More targeted to specific pest Generally nontoxic to humans, mammals, and bees.	*Narrower target range*
Specific mode of action Generally affect only the target pest and closely related organisms, in contrast to broad spectrum pesticides.	*Specific mode of action* Care to choose the product that targets the pest you need to control.
Manage rather than eradicate Maintain ecological balance. Suppress, rather than eliminate, a pest population.	*Slower acting* The time from exposure to morbidity and death of the target insect may be 2 to 10 days.
Often effective in very small quantities and often decompose quickly Thereby resulting in lower exposures problems.	*Costs and availability* Generally more expensive than synthetically derived insecticides. Lack of sales and problems associated with providing consistent product.
Minimal impacts on plants Most botanical insecticides are not harmful to plants when applied according to the label directions.	*Lack of efficacy data* Insufficient data exist on botanical insecticides, both in terms of effectiveness and chronic toxicity.

A general classification of biopesticides is based in three different categories according to the type of active ingredient used: (i) plant-incorporated protectants, (ii) microbial pesticides, and (iii) biochemical pesticides [13]. In general, there are significant differences in the mode of action between microbial and biochemical (Table 2) [14]. The majority of the biopesticide market, approximately 90%, are living organisms. These include biofungicides (*Trichoderma*), bioherbicides (*Phytophthora*) and bioinsecticides (*Bacillus thuringiensis*).

A more recently introduced term is biocontrol agents instead of biopesticide, which are classified into four groups: (i) macrobials, (ii) microbials, (iii) natural products, and (iv) semiochemicals (insect behavior-modifying agents). Among all the biocontrol agents, the most important products are microbials (41%), followed by macrobials (33%), and finally other natural products (26%) [15].

In addition to being categorized by the active ingredient, biopesticides can be categorized by the target pest, such as insecticides to manage insect populations and fungicides to manage fungus.

The need for more environmentally-friendly forms of pesticide is therefore greater than ever. Therefore, biopesticides have gained increasing importance due to their potential in developing environmentally friendly and safe approaches and tactics for pest management, in particular for the development of products that could replace synthetic chemical pesticides [16].

Table 2 Categories of biopesticides [19].

Types	Actives Ingredients	Mode of action	Examples [13]
Plant-incorporated protectants	Genetic material added to the plant, which is commonly known as a transgenic crop or a genetically modified organism.	Plants themselves can produce the proteins and protect themselves from insects without any external pesticide.	Coat protein gene of *Plum Pox* virus *Bacillus thuringiensis Vip3Aa20, Cry1A.105* and Cry2Ab2
Microbial pesticides	Naturally occurring or genetically controlled microorganism. The active ingredient can be either the spores or the organism itself.	Relatively specific for its target pests. Act by exploitation, competition, antibiosis, lysis and/or induced resistance.	*Ulocladium oudemansii* *Bacillus thuringiensis* *Beauveria bassiana* *Trichoderma asperellum* *Trichoderma gamsii*
Biochemical pesticides	Chemicals either extracted from natural sources or synthesized to have the same structure and function as the naturally occurring chemicals. They control pests by non-toxic mechanisms.	Act by contact, ingestion, systemic action, suffocation and/or attraction/repulsion. Include substances that interfere with growth or mating, such as plant growth regulators, or substances that repel or attract pests, such as pheromones.	They include substances, such as insect sex pheromones, as well as various scented plant extracts, or fatty acids. Cold pressed *Neem Oil* Extract of *Chenopodium ambrosioides* Saponins of *Quillaja saponaria*

The term “natural” conveys a sense of wholesomeness or safety, however it is important to indicate that based on the LD_{50}, a number of registered botanicals are toxic to fish, beneficial insects and mites, and mammals. In fact, several botanical insecticides have a lower LD_{50} than the synthetically derived insecticides carbaryl (Sevin) and malathion (Table 3). Although naturally occurring insect toxins are extracted from plants, “natural” does not necessarily imply “safe” or “nontoxic.” For example, arsenic, strychnine, lead, mercury, nicotine, and other similar compounds used historically as pesticides technically qualify as natural. Today no one considers these compounds wholesome or safe. In most cases, botanical insecticides are less toxic to humans than synthetically derived insectides [17]. For example, *Nicotiana tabacum* is the most toxic of the botanical insecticides, with an LD_{50} between 50 and 60 mg/kg. It is extremely harmful to humans. Nicotine, a fast-acting nerve toxin, works as a contact poison.

Biopesticides offer an environmentally sustainable approach to increase crop production and health. There is a tremendous amount of work and research occurring in this field. The topic of genetically modified organisms is a broad topic that warrants its own investigation, and will not be covered in this report. In this chapter, we intend to present an overview of the most significant advances described in the latest literature concerning the challenges and opportunities for the development of biopesticides. We mainly focus on Biochemical pesticides, including the progress achieved in the discovery process of new potential biopesticides. For the purposes of this report, biopesticides have been categorized in a manner similar to that used by the EPA.

Table 3 Ranking of pesticide with each other and commonly used synthetically derived insecticides, based on their toxicity rating (oral LD_{50}).*

Common Name	Active compounds	Acute Oral LD_{50}	Acute Dermal LD_{50}	Bees	Type of pesticide
Allium sativum	Allicin	3034	–	Non-toxic	Insecticide
Bacillus thuringiensis	–	>5,000	>2,000	Non-toxic	Insecticide
Capsicum annum	Capsaicin	148-161	>512	Toxic	Insecticide
Citronella oil	Citronellal, geraniol	7200	4700	Low-toxic	Insecticide, Herbicide
Citrus oil	Limonene, linalool	4,000-5,000		Low-toxic	Insecticide
Clove essential oil	Eugenol	2650	–	Low-toxic	Insecticide, Herbicide
Kinoprene	–	4,950	9,000	Non-toxic	Insecticide
Neem oil	Azadirachtin, dihydroazadirachtin	4,200	2,000	Low-toxic	Insecticide, Acaricide, Fungicide

Nicotiana tabacum	Nicotine	55	–	High-toxic	Insecticide
Pyrethrin	Esters of chrysanthemic and pyrethric acid	1,500	>1,800	High-toxic	Insecticide, Acaricide
Rotenone	Rotenone, deguelin	350	940	High-toxic	Insecticide, Acaricide
Ryania speciosa	Ryanodine, 9,21-dehydro ryanodine	750	–	Low-toxic	Insecticide
Sabadilla	Cevadine, veratridine	5,000	–	High-toxic	Insecticide
Warfarin	–	3	–	Non-toxic	Rodenticide
DDT	–	113	2,510	High-toxic	Insecticide
Malathion	–	2,800	4,100	High-toxic	Insecticide
Carbaryl	–	246-283	4,000	High-toxic	Insecticide

* LD_{50}: It is the amount of a material, given all at once, which causes the death of 50% of a group of test animals. LD_{50}values are expressed as milligrams per kilogram (mg/kg), which means milligrams of chemical per kilogram of body weight of the animal.

2. Living organisms as Insecticides

Microbial products may consist of the organisms themselves and/or the metabolites they produce to control of pest insects, plant pathogens and weeds. Over 400 species of fungi and more than 90 species of bacteria which infect insects have been described. These microorganisms, are legally considered biopesticides and are regulated as such [13]. In the case of although multicellular organisms such as nematodes, they are not regulated as pesticides. The advantage to using biological products is their higher selectivity, therefore they are lower or no toxic to non pathogenic organism, to wildlife, humans, and other organisms not closely related to the target pest, in comparison to conventional chemical pesticides. A limitation of several types of microbial insecticides is that heat, desiccation or exposure to ultraviolet radiation reduces their effectiveness, and consequently, proper timing and application procedures are especially important for some products [18]. Bacteria, fungi, oomycetes, viruses and protozoa are all being used for the biological control. In Table 4 we described some special characteristics of these types of microbial insecticides.

Table 4 Types of microbial insecticides [19-21].

Bacteria	
Uses	Bacterial biopesticides are the most common and cheaper form of microbial pesticides. They are effective against a wide spectrum of plant diseases, nematodes, insects, and weeds.
Mode of action	Must be eaten to be effective. Bacteria disrupt the digestive system by producing endotoxins that are often specific to the particular insect pest.
Examples	*Bacillus sphaericus* used to control certain mosquito species. *Bacillus subtilis* and *Bacillus pumilus* increase yield and prevent plant diseases by outcompeting plant pathogens in the rhizosphere, producing anti-fungal compounds.
Fungi	
Uses	Useful to control plant diseases caused by other fungi, bacteria or nematodes, as well as some insect pests and weeds. They may be applied in the form of conidia or mycelium which sporulates after application. Very useful for insecticide resistant management.
Mode of action	The mode of action is varied and depends on both the pesticidal fungus and the target pest. One advantage of fungal biopesticides is that they do not need to be eaten to be effective.
Examples	*Trichoderma spp.* colonize plant roots, without harming the plant. It can out-compete pathogenic fungi for food and space, and in the process can stimulate plant host defenses and affect root growth. *Beauveria bassiana* is effective in controlling troublesome crop pests such as aphids, thrips and whitefly – even chemical pesticide-resistant strains such as Q-Biotype Whitefly. Have been reported the use of *Metarhizium anisopliae* against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes.
Protozoa	
Uses	Protozoan pathogens, single-celled eukaryotic organisms, naturally infect a wide range of insect hosts.

Mode of action	Must be eaten to infect an insect. They enter the insect via the gut wall, spreads to various tissues and organs, and they multiply, sometimes causing tissue breakdown and septicemia. One important consequence is a reduction in the number of offspring produced by infected insects.
Examples	*Nosema locustae* infects at least 90 species without affects to humans and other mammals, as well as the over 250 natural predators of grasshoppers. *Nosema pyrausta* infects several insect species, including European corn borer, for which it can be an important natural control. *Vairimorpha necatrix* has a wide host range among caterpillar pests, including corn earworm and European corn borer, various armyworms, fall webworm, and cabbage looper.
Viruses (baculoviruses)	
Uses	Baculovirus is the main virus that is commercially used. It is a special family of naturally-occurring viruses, to which about 100 insects and some related arthropods are susceptible. They are very specific. Baculoviruses used consist of DNA surrounded by a protein coat (nucleocapsid), which is itself embedded in a protein "microcapsule" or occlusion body (OB) that provides some protection from degradation in the environment.
Mode of action	Upon ingestion by a susceptible caterpillar, OBs are dissolved within the alkaline midgut, releasing nucleocapsids that infect the cells lining the midgut. The viral DNA replicates in the nuclei of the host cells and then spreads throughout the body of the larvae. The infected insect stops feeding within a few days, dies and disintegrates.
Examples	The granulovirus of the codling moth *Cydia pomonella*, it is the active ingredient of about a half-dozen products sold worldwide, and limits codling moth populations and damage in pome fruits while preserving beneficial insects and minimizing chemical residues. Preparations of *Heliothis zea* nucleopolyhedrovirus infects many species of *Helicoverpa* and *Heliothis* genera. These products provided control of not only cotton bollworm, but also of pests belonging to these genera attacking soybean, sorghum, maize, tomato and beans. *Anticarsia gemmatalis* nucleopolyhedrovirus control the velvetbeen caterpillar in soybean.
Yeast	
Uses	*Cryptococcus* and *Candida* species have been investigated for their usefulness in controlling plant diseases.
Mode of action	Works primarily through competition for nutrients and pre-colonization of plant wound sites. It exist evidence that it produces enzymes that can degrade fungal cell walls and stimulate plant host defence pathways in freshly harvested fruit.
Examples	*Candida oleophila* (strain O) is an effective biopesticide for the control of post-harvest fruit rots. It is applied to apples and pears after harvest — but before storage — to control particular fungal pathogens. The yeast serves as an antagonist to fungal pathogens such as gray mold (*Botrytis cinerea*) and blue mold (*Penicillium expansum*), which cause post-harvest decay.

We refer to data published annually by the State of California's Department of Pesticide Regulation to discuss the extent to which each of the more important botanical insecticides are used [22]. At present *Bacillus thuringiensis* is the most studied entomopathogenic species and some of its crystal producing strains have certainly represented the main active substances used for the microbial pest management during the last decades. Additional microorganisms as *Beauveria bassiana* have limited use in various countries (table 5).

Table 5 Microbial treatment most often used against insect pests.

Bacillus thuringiensis (Bt) [1, 23-24]	
Active compounds	Bt is a ubiquitous Gram-positive, rod-shaped and sporulating bacterium that synthesize crystal (Cry) and cytolytic (Cyt) toxins, (also known as δ-endotoxins), at the onset of sporulation and during the stationary growth phase as parasporal crystalline inclusions.
Mode of action	Once ingested by insects, these crystals are solubilized in the midgut, the toxins are then proteolytically activated by midgut proteases and bind to specific receptors located in the insect cell membrane leading to cell disruption and insect death. The δ-endotoxins are host specific and can cause host death within 48 h.
Uses	The most world-wide used biopesticide. Microbial Bt biopesticides consist of bacterial spores and δ-endotoxins crystals mass-produced in fermentation tanks and formulated as a sprayable product. It feeds on the larval stages of insect pests such as mosquitoes, Colorado potato beetles, and cabbage loopers. It has been used for pest management on fruit and vegetable crops such as maize, soya bean and cotton.
Advantages	It does not harm vertebrates and is safe to people, beneficial organisms and the environment. High level of selectivity, so different strains of Bt are effective against specific pests. For example, *Bt. var. kurstaki* kills caterpillars, while *Bt. var. israelensis* is for mosquitoes and other fly larvae. Useful where resistance to synthetic chemical insecticides is a problem.
Disadvantages	*B. thuringiensis* products can be sensitive to sunlight, giving them a very short period of effectiveness, so multiple applications are often needed for adequate management of the pest. Bt is only effective when ingested.
Total pesticide used in California (2014)	*Bt (berliner), subsp. kurstaki*, strain SA-11: 80,311 lb (36,428 Kg) *Bt, subsp. aizawai*, strain abts-1857: 48,784 lb (22,128 Kg) *Bt, subsp. israelensis*, strain am 65-52: 42,746lb (19,389 Kg) *Bt*, *subsp. kurstaki*, strain abts-351: 111,273lb (50,472 Kg)

Beauveria bassiana[1, 25-26]	
Active compounds	The key secondary metabolites produced by *Beauveria bassiana*, a soilborne fungus, are beauvericin, bassianolide, bassianin, tenellin and cyclosporin A.
Mode of action	*B. bassiana* can be applied as a spore. Once the spores have contact with the insect exoskeleton, they grow hyphae (long, branching vegetative appendages) that secrete enzymes, which in turn dissolve the cuticle (outermost layer of the skeleton). These fungal hyphae then grow into the insect, feed on its body tissue, produce toxins, and reproduce. It takes up to seven days for the insect to die.
Uses	Effectively to control thrips, aphids, whitefly, caterpillars, beetles, and subterranean insects like ants, spider mites and termites.
Advantages	It does not harm vertebrates and is safe to people, beneficial organisms and the environment. The strain used and sold commercially does not affect honey bees
Disadvantages	*B. bassiana* can be sensitive to sunlight, giving them a very short period of effectiveness. Its spores will infect many non target beneficial insects too. Toxic metabolitesof *B. bassiana* may enter the plants. In minor cases conidia of *B. bassiana* have allergenic potential.
Total pesticide used in California (2014)	*Beauveria bassiana* strain GHA: 2,746lb (1,246 Kg)

3. Plant Extracts as Natural Insecticides

In the search for alternative solutions to crop protection problems, the interest in plants extracts has increased. Throughout evolution plants have developed an effective defence system against microbial attack or insect/animal predation, including the production of low molecular mass secondary metabolites with antimicrobial activity, which are synthesized *de novo* after stress and are collectively known as phytoalexins, which have toxic, repellent, and/or antinutritional effects on pest [27-28]. This plethora of chemical-defensive compounds include alkaloids, phenolics and terpenoids, and could be used to combat insect pests and disease pathogens. Their mechanisms of action can vary, especially when the activity is due to a complex mixture of compounds that can be toxic or repellent to the target organisms and cause developmental changes including sterility, reduced growth, and altered behavior. Till date, over 2000 plant species have been known to produce secondary metabolites of value in biological pest control programs, and many of these plants are used by farmers in developing countries. Only a small percentage of plants have been screened for pesticidal activity, thus potentially useful biological compounds remain undiscovered [29-30].

Of particular interest are essential oils, which are generally composed of complex mixtures of mono and sesquiterpenes. In the nature those compounds plays an important role in the protection of the plants against bacteria, fungi, virus, insects and others herbivores. For these reasons, certain essential oils are considered to be an alternative means of controlling many harmful insects, especially against small, soft-bodied insects and mites that are immobile or slow-moving (e.g., aphids, scales, leafhopper nymphs, whiteflies). Essential oil products are generally considered as broad spectrum because of the presence of several active ingredients that operate through several modes of action, including larvicidal and antifeeding, inhibit molting and respiration, reduce growth and fecundity, and display phototoxicity [31-33].

Humans have made use of botanical insecticides to control pests for hundreds of years. These products can be marketed in one of three ways: (1) preparations of the crude plant material, ground into a dust or powder; (2) extracts from plant resins, formulated into liquid concentrations; and (3) isolation of the pure chemicals obtained from plants by extraction or distillation.

We refer to data published annually by the State of California's Department of Pesticide Regulation to discuss the extent to which each of the more important botanical insecticides are used [22]. In the present there are four major types of botanical products used for insect control (pyrethrum, rotenone, neem, and essential oils), along with three others in limited use (ryania, nicotine, and sabadilla). Additional plant extracts and oils (e.g., garlic oil, *Capsicum* oleoresin) see limited (low volume) regional use in various countries (table 6) [34].

Table 6 Alternative plant extracts options for control of insect pests.

Neem-based insecticides [35-36]	
Active compounds	Limonoids, mainly azadirachtin A and B, obtained from different parts of the plant (mainly the seed) of the neem tree (*Azadirachta indica* A. Juss). The best solvents for the extraction of azadirachtin A are water and methanol.
Mode of action	Antifeedancy, fecundity suppression, ovicidal and larvicidal activity, growth regulation and repellence, also at low dosages. Azadirachtin reduces the level of the insect hormone Ecdysone, which affect corpus cardiacum and block reproductive and growth processes in most insects causing sterility in females and degenerative changes in male testis due to disturbance in insect metabolism.

Uses	For more than 400 insect pests of medical, veterinary and agricultural importance including pests species belonging to Lepidoptera, Diptera, Coleoptera, Homoptera and Hemiptera. Range of crops.
Advantages	Non-toxic to humans and animals, including useful insects like bees, exhibits fewer chances of resistance, due to its multiple mode of action on insects, and have no residual effect on agricultural products. In most tests neem products performed equally or sometimes better than synthetics. Neem based pesticides are easy to prepare, cheap and highly effective.
Disadvantages	Limited outdoor crop studies.
Total pesticide used in California (2014)	Clarified hydrophobic extract of neem oil: 196,906 Pounds (89315 Kg) Margosa Oil (Obtained from the fruits and seeds of neem: 22,547 Pounds (10227 Kg) Azadirachtin: 3,999 Pounds (1814 Kg)
Garlic essential oil [37-38]	
Active compounds	The principal biologically active compound produced by garlic (*Allium sativum*) is allicin, a sulphur containing (thiosulphonate)
Mode of action	Some studies have proved that garlic oil has fungicidal, repellent, insecticidal, nematicidal, and antibiotic properties.
Use	It is used on a wide range of pests, including aphids, thrips, leafhoppers and caterpillars. Crops: avocados, citrus, ornamentals.
Advantages	Garlic exhibits antibacterial, antifungal, amoebicidal and insecticidal qualities. Garlic is not persistent in the environment.
Disadvantages	Toxic to bees and fish. Degradation rapid due to sunlight and ultraviolet light. Thus, it is not recommend as an all-purpose spray for outdoor use. Limited studies.
Total pesticide used in California (2014)	Garlic essential oil: 1,392 Pounds (631 Kg)
Chenopodium ambrosioides near ambrosioides[39-40]	
Active compounds	Terpenes: α-terpinene, d-limonene and p-cymene
Mode of action	The active ingredient is lipophilic; attracted to the oily outer surfaces of target pests, and works to kill target pests in three ways: 1. Collapses trachea causing asphyxiation; 2. Destroys cuticle layer causing desiccation; 3. Anti-feeding properties.
Use	Controls soft-bodied sucking pests including thrips, whiteflies, aphids, mites, leaf hoppers, leafminers, and psyllid, in high-value fruits and vegetables. It also deters feeding and reduces the spread of viruses.
Advantages	Resistance development reduced/unlikely due to three different modes of action. Active against all lifecycle stages – eggs to adults. Safe for workers, the environment, and neighbours. Field trials confirm safety on Bees (*Apis mellifera*).
Disadvantages	Good coverage is essential. Nil/Limited outdoor field studies.
Total pesticide used in California (2014)	*Chenopodium ambrosioides* near ambrosioides essential oil: 17,504 Pounds (7940 Kg)
Canola oil [41]	
Active compounds	Refined vegetable oil obtained from the seeds of four species of rape plants of the family *Cruciferae* (mustard family): *Brassicanapus*, *B. juncea*, *B. rapa* and *B. campestris*.
Mode of action	Repels insects by altering the outer layer of the leaf surface or by acting as an irritant.
Use	Citrus, corn, fruit trees, nut trees, sugar beets, soybeans, tomatoes, vegetables, figs, melon, olives, small fruits, alfalfa, bedding plants, ornamentals, and houseplants.
Advantages	The oil is biodegradable and leaves little residue and does not taint the crops.
Disadvantages	Application when temperatures are high (above 30–35°C) and/or humidity is low may cause leaf scorch and interfere with plant respiration. Reapplication may be necessary depending on weather conditions. Limited outdoor crop studies.
Total pesticide used in California (2014)	Canola oil: 34 Pounds (16 Kg)

	Oriental Mustard Seed Allyl isothiocyanate [42-43]
Active compounds	Seeds of brown or oriental mustard (*Brassica juncea*),with high concentrations of sinigrin (ally lglucosinolate, *S*-glucopyranosyl thiohydroximates).
Mode of action	The glucosinolates present in the *Brassica* species are hydrolyzed, upon contact with water, by thioglucosidases called myrosinases to isothiocyanates, which have been proven toxic against insects, fungi and nematodes.
Use	Wide spectrum of anti-microbial effects and repellents against certain species of insect. Insecticidal. Nematicidal activity on *Heterodera glycines*, *Pratylenchus neglectus*, *Heterodera schachtii*, *Pratylenchus penetrans*, *Meloidogyne incognita*, *Meloidogyne hapla* and *Caenorhabditis elegans* in the laboratory; and on *Pratylenchus penetrans* on sweet corn in a greenhouse.
Advantages	The oil is biodegradable and leaves little residue and does not taint the crops.
Disadvantages	The effectiveness of these practices in suppressing nematode populations in soil has not been conclusive.
Total pesticide used in California (2014)	-
	Pyrethrum [44-45]
Active compounds	Oleoresin extracted from the dried flowers of *Tanacetum cinerariaefolium*. Two pyrethrins are most prominent, pyrethrin-I and pyrethrin-II. The pyrethrins have another four different active ingredients, Cinerin I and II and Jasmolin I and II.
Mode of action	They are contact poisons which induce a quickly neurotoxic effect in insects. They delay the closure of voltage-gated sodium ion channels in the nerve cells of insects, resulting in repeated and extended nerve firings. This hyperexcitation causes the death of the insect due to loss of motor coordination and paralysis. The mechanism is qualitatively similar to that of DDT and many synthetic organochlorine insecticides.
Use	Broad spectrum of insect pests of medical, veterinary and agricultural importance.
Advantages	They are one of least toxic domestic insecticides available (rat oral acute LD_{50}value~1500 mg kg^{-1}). Broad spectrum of insecticides.
Disadvantages	Pyrethrin is extremely toxic to aquatic life. Natural pyrethrins are highly fat soluble, but are easily degraded and thus do not accumulate in the body. These compounds are toxic, and affect their feeding behavior. Pyrethrins are degraded by the combination of sunlight and air, and therefore present low persistence.
Total pesticide used in California (2014)	Pyrethrum accounted for 80% of all botanicals used that year, but only 27% of that amount was used in agriculture.
	Heliopsis longipes [46-47]
Active compounds	Alkamides as *N*-isobutil-2*E*,6*Z*,8*E*-decatrienamide and *N*-isobutil-2*E*,6*Z*,8*E*-decatrienamide
Mode of action	Similarly to pyrethrins, it is a potent voltage-dependent blockers of sodium channel, with paralyzing effects.
Use	Control populations of insect vector transmitters of several diseases that affect the human health as *Anopheles albimanus* (LC_{50} 4.24mg/L) and *Aedes aegypti (*LC_{50} 7.38 mg/L).
Advantages	Non-toxic to humans and animals, including useful insects like bees.
Disadvantages	It is too unstable to be used as products directly. Limited outdoor crop studies.
Total pesticide used in California (2014)	-

4. Process to identify, evaluate, and develop new biopesticides from natural products

Development of new and useful biopesticides has continued to increase rapidly since the mid-1990s. Cantrell *et al*. [48] found that from the years 1997–2010, 277 new active ingredients (NAI) were registered as conventional pesticide or biopesticide for the United States Environmental Protection Agency (EPA). When examining conventional pesticides and biopesticides combined, and considering that natural products, synthetic natural derived, and biological all have origins from natural product research, it can be argued that their combined impact with the EPA from 1997 to 2010 accounted for 69.3% of all NAI registrations. It shows clearly that natural products play an important role in discovery and development of new products. However, nature's diversity has not been efficiently explored for discovery of new natural-product pesticides.

Different approaches to discovery and development of pesticide compounds using higher plants can be distinguished: a. random selection followed by chemical screening; b. random selection followed by one or more biological assays; c. follow-up of biological activity reports; d. follow-up of ethnobotanical information [49]. Once the interest lignocellulosic materials is chosen, new active substances should be isolated and identificated. Bioassay-guided fractionation has proven successful as a well-established platform to isolate and characterize active constituents present in natural product extracts; however, sometimes such an approach requires multiple chromatographic steps and large amounts of biological material. It is necessary to develop new technologies to accelerate this phase. Process such as automated separation techniques, high-throughput screening and combinatorial chemistry are revolutionizing lead compounds discovery. Metabolomics offers a new way of studying complex molecular problems and is particularly applicable for natural products research. [50].

The most common factors affecting extraction processes are matrix properties of the plant part, solvent, temperature, pressure and time. Traditional techniques such as solid-liquid or Soxhlet extractions have been used for many decades, but they are time-consuming and require relatively large quantities of solvents. Also, due to the common extractive steps used by these techniques, including heating, boiling, or refluxing, a loss of metabolites due to ionization, hydrolysis, and oxidation occurs during the procedure [51]. In recent years, some new more selective and environmentally friendly techniques were successfully proposed for extraction of metabolites. Non-conventional methods, which are more environmental friendly due to decreased use of synthetic and organic chemicals, reduced operational time, and better yield and quality of extract. They include ultrasound, pulsed electric field, enzyme digestion, extrusion, microwave heating, ohmic heating, supercritical fluids extraction, and accelerated solvents have been studied [52].

After the active ingredients with crop protection properties have been purified, and its structure have been determined by spectroscopic techniques, their biological activity must be supported by numerous bioassays both *in vitro* and *in vivo*. *In vivo* screens give an early realistic read-out of efficacy in the practical context and *in vitro* tests have particular utility in unearthing new mode of action targets. Criteria that distinguish good bioassays are: reproducibility; linearity over a reasonable dose or concentration range; and predetermined endpoints. However, after the new compound has demonstrated in vitro activity against the insect, other numerous criteria must be satisfied. It is essential to determinate the chemical, toxicological, mode of action and environmental properties of new compounds in the development process, and before trying to commercialize them [53].

The final step is scale-up of the production. Botanical extracts are well-recognized sources of active molecules. However, botanical extracts are not always well accepted because of issues concerning active compound standardization, and quality control. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is of great interest to develop well-characterized extracts in order to achieve biochemical and functional consistency between batches [54]. However, several problems not applicable to synthetic drugs often influence the quality of herbal drugs, for instance: Herbal drugs are usually mixtures of many constituents; the active principle(s) is (are), in most cases, unknown; selective analytical methods or reference compounds may not be available commercially; plant materials are chemically and naturally variable; chemo-varieties and chemo cultivars exist; the source and quality of the raw material are variable. The deployment of modern analytical tools in testing the various quality parameters for an effective quality control herbal product cannot be over emphasized. The assurance of the safety and efficacy of an herbal drug requires monitoring of the quality of the product from collection through processing to the finished packaged product [55].

Finally, it is important to note that the formulation of the pesticide determine the overall efficacy, increase product stability and viability of the chosen substance once applied in field conditions. In order to choose the correct formulation one must take into account several factors such as physicochemical and biological properties, mode of application, the crop to be treated and economic considerations. Several commonly used biopesticide formulations include dry and wet powder, granules and pellets. Granules can protect the active agent from desiccation and also provide basic food for the agent, however powder is easy to apply by suspending it in water and also can cover a wide area of application.

At the present it is fundamental the development of new formulations to improve the potency, stability, and the safety of these systems to humans and the environment. The use of nanotechnology and controlled-release formulations could offer a way of developing new formulations with smaller quantities of active compound to be used more effectively with a minimization of environmental damage [56-57].

5. Conclusions and new opportunities for developing biopesticides

Today, biopesticides are an emerging technology. Worldwide there are about 1400 biopesticide products being sold, which are based on 68 active substances registered in the EU and 202 in the USA, with global market of approximately $3 billion in value, with a compound annual growth rate (CAGR) of 8.64% (compared with 3% for synthetic pesticides) which is expected to produce a global market of $10 billion by 2017. It is clear that the development of the biopesticides market depend on economic terms: a. Company producing these products should have profits; b. The comparative efficacy and the cost:benefit ratio of natural and synthetic insecticides should be favorable to the first one [1, 58]. We show in the table 7 various features which could be fundamental for the development of this technology.

Table 7 Features which could be fundamental for the development of this technology.

Advantages
Lower development price [58]. The costs associated with the development of a novel synthetic pesticides typically requires $250 million and nine years of research and regulatory approval, while a biopesticide needs less than $10 million and four years for the same process.
Emerging resistance in insects to conventional agrochemical insecticides [59]. Resistances to pesticides currently available are wide spread, while it takes longer for insects to develop a resistance to biopesticides because they have new and multiple modes of action.
Disadvantages
Regulatory barriers to biopesticide commercialization [1]. It is very expensive to prepare the biopesticide registration data portfolio, which includes information about mode of action, toxicological and ecotoxicological assays or host range testing, among others.
High competition with synthetic pesticides. It is fundamental to carry out *in vitro*, *in vivo* and field tests, to demonstrate the effectiveness of the product.
Highly specific activity. These products have a smaller market than those for products with broad spectrum activity, as chemical substance or some biopesticides (e.g. *Bacillus thuringiensis*, azadirachtins, etc.). This problem often forces biopesticide use in conjunction with conventional agrochemicals.

There is an increasing global search for environment friendly agrochemicals. However, the biopesticide production must overcome important problems which include slower pest control and higher manufacturing costs compared with conventional agrochemicals, as well as regulatory problems for their commercialization. Production technology of biopesticides should be improved, along with the implementation of quality systems for the standardization and uniformity of the process. The commercial biopesticide must be reliable, specific, indigenous, and replicable in its activity. Moreover, extensive research should be conducted in the formulation of the product to prevent degradation in the environment and to extend the shelf life of the biopesticide. Topics of current scientific interest in formulation involves lyophilized preparations of biopesticides, or the development of oil-in-water microemulsions as a nano-pesticide delivery system, in order to improve the field persistence, reduce the use of organic solvent and increase the dispersity, wettability and penetration properties of the droplets. Developed formulation should allow for long storage periods, and it should be compatible with crop production practices and equipment.

In conclusion, if the industry can obtain high quality products with the ability to act for long time in field conditions, the bioinsecticides will be able to compete with the agrochemical insecticides currently available and gradually overtake the market.

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