bioresources.com

Biopesticides from Natural Products: Current Development, Legislative Framework, and Future Trends

Juan José Villaverde,* Pilar Sandín-España, Beatriz Sevilla-Morán, Carmen López-Goti, and José Luis Alonso-Prados

The importance of biomass as a source of chemicals, biofuels, and energy is widely accepted. Currently, the attention is mainly focused on the valorisation of by-products from lignocellulosic materials. Chemical compounds derived from plants and microorganisms that provide good protection for crops against weeds, pests, and diseases (biopesticide active substances) have been used to formulate pesticides. Their use is increasingly encouraged by new pesticide regulations that discourage the use of harmful active substances. This article reviews the current and future situation of biopesticides, especially natural chemical products, and focuses on their potential within the European pesticide legislative framework. Moreover, this article highlights the importance of the different modes/mechanisms of action of the active substances obtained from natural sources, the role of chemistry in biopesticide development, and how the adoption of integrated pest management practices contributes to a greater trend towards biopesticides.

*Contact information: Plant Protection Products Unit, DTEVPF, INIA, Ctra. de La Coruña, Km. 7.5, 28040 Madrid, Spain; *Corresponding author: juanjose.villaverde@inia.es*

INTRODUCTION

The concept of an ideal pesticide has evolved greatly during the second half of the 20th century. In the early 20th century, pesticides were used with a low level of control and without harmonized criteria because compounds efficacy was prevailing. Thus, many pesticides that are currently removed from the market because of high toxicity and persistence were used extensively with unsuitable application methods developed during World War II, such as airplane application (Howard 1935). This application caused many cases of acute or chronic toxicity (Hunt and Bischoff 1960), and other adverse effects such as an increased resistance in the target species (Mouches *et al.* 1986), the replacement of target species with more harmful resistant species (Regnault-Roger *et al.* 2005), and contamination of different environmental compartments (Ellgehausen *et al.* 1980; Leduc *et al.* 1987). When these problems appeared, authorities demanded that these pesticides be used appropriately, and they revised pesticide legislation and created agencies devoted to the protection of human and animal health and the environment from risks associated with pesticides (*e.g.*, the United States Environmental Protection Agency (USEPA) (USEPA 2015) and the European Food Safety Authority (EFSA) (European Commission 2002)). Currently, the concept of the ideal pesticide has significantly changed to include (i) a high selectivity to target species but a minimal toxicity to non-target organisms, (ii) a high effectiveness at a low application rate, and (iii) a low environmental persistence (readily

degradable) to avoid bioconcentration and biomagnification within the food chain and to prevent the development of resistances. Thus, there is a need for new active substances that effectively fight pests but have minimal impacts on humans, animals, and the environment.

Natural products and living organisms have a long history as crop protection agents. Pesticides are usually referred to as "biopesticides" when they are formulated using these kind of substances. In the last decade, the use of the term biopesticide has increased considerably (Fig. 1), but at the legislative level, there is not a global agreement on this term. Both the United States and European Union have well-developed pesticide legislation to protect human and animal health and the environment, in a comparably sized market and bring new active substances and formulations to market faster. The USEPA defines biopesticides as formulations derived from natural materials such as animals, plants, bacteria, and certain minerals (USEPA 2015). At the European level, there is no specific designation, although biopesticide active ingredients are generally but not always defined as "low risk" and "basic" substances.
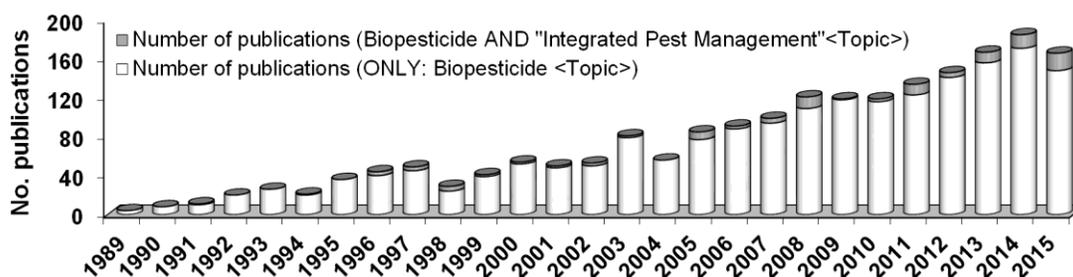

Fig. 1. Evolution of the term "biopesticide" in the scientific literature (Web of Science 2015)

At the beginning of this century, about 1400 biopesticide products were sold around the world, accounting for about 2.5% of the total pesticide market (Marrone 2007; Chandler *et al.* 2012). The demand for biopesticides is expected to grow at a faster compound annual growth rate of 16.1% (compared with 3% for synthetic pesticides) from 2012 to 2017, which will produce a global market of $5.2 billion in 2017 (Lehr 2010; Market and Markets 2012). Currently, North America consumes about 40% of global biopesticides, with Europe, Oceania, and Latin America accounting for 20, 20, and 10%, respectively (Leng *et al.* 2011). Some of these regions (*i.e.*, Europe and North America) are important markets for the promotion of these products, with fast growing and modern control systems that support and regulate their use. However, contrary to common belief, biopesticides usually generate residues that may be harmful. The biopesticide impact on humans, animals, and the environment is only reduced by the implementation of strategies that diminish and optimize their usage.

Since adoption of the Council Directive 91/414/EEC (European Commission 1991), which was later replaced by the current Regulation (EC) No. 1107/2009 (European Commission 2009b), any plant protection product (henceforth pesticide) must pass a rigorous evaluation process that proves that it does not pose risk before being marketed in any Member State of the European Union. The safe use of pesticides should also be ensured. Thus, numerous legislative measures have been adopted, such as the European Directive 2009/128/EC for sustainable use of pesticides (European Commission 2009a). This directive addresses a gap in the European legislative outline related to pesticide

management, providing clear references on managing pesticide field applications to reduce their use and to prevent potential environmental contamination.

In the European Union, public institutions such as KEMI in Sweden, ANSES in France, CRD in the UK, or INIA in Spain evaluate scientific documentation submitted by companies within the agrochemical sector for the approval of the pesticide active substances and authorization of their commercial formulations.

This review analyzes the impact of biopesticide active substances on the development of new pesticide formulations and their future use in the European market, taking into account the current legislative framework. Some case assessments of biopesticide active substances under European Union Regulation are also discussed. This review has been focused on natural chemical compounds derived from plants and microorganisms. Among microorganisms, special attention is given to those that use cheap raw materials, such as residues from agroforest industries, decaying vegetation, among others, as substrates in the production of bioactive substances (Zhang *et al.* 2013). Both plants and microorganisms as sources of chemicals for crop protection open promising perspectives within the biorefinery concept.

RESULTS AND DISCUSSION

Modes and Mechanisms of Action to Avoid Pest Resistance

Plants have been important sources of natural pesticides for centuries; for example, tobacco plants such as *Nicotiana tabacum* are sources of the alkaloid nicotine (Dayan *et al.* 2009). Microorganisms are also used to produce biopesticide active substances from lignocellulosic materials. Two species of the bacterial genus *Saccharopolyspora* produce spinosyns through the fermentation of carbohydrates or vegetative media (Kirst *et al.* 1991; Lewer *et al.* 2009). Some common natural chemical compounds are included in Table 1 and Table 2.

There are natural pesticides for the control of plant pathogens (bacteria or fungi), insects, and weeds, as well as natural products for the management of mammals and birds (repellents) (Dayan *et al.* 2009). The biological activities and mechanisms of action of these compounds are extremely variable (Aliferis and Jabaji 2011). This phenomenon reflects the fact that several of these natural products are derived from secondary metabolism pathways, which allow plants to protect themselves from their natural enemies (Chandler *et al.* 2012). Thus, it is likely that natural products have biological activity against other organisms.

The mode of action (MoA) describes the specific biochemical interaction (target or site of action) to which bioactivity is attributed (FRAC 2015; HRAC 2015; IRAC 2015). MoA is sometimes differentiated from mechanism of action (Aliferis and Jabaji 2011); however, both terms should be used without distinction (Casida 2009). The MoA for these compounds is not always understood; examples in the literature include, saponins (Geyter *et al.* 2007), momilactone B (Kato-Noguchi *et al.* 2013), azadirachtin (IRAC 2015), cinnamaldehyde (Kang *et al.* 2007; Brown 2013), acetic acid (Reynolds 1974), and pelargonic acid (HRAC 2015). In some instances, it is likely that they have multiple activities. Understanding MoA is fundamental because it clarifies the most effective and sustainable pesticide in the pest resistance management strategy.

bioresources.com

Table 1. Examples of Natural Chemical Compounds with Activity against Bacteria, Fungi, and Weeds

Active Substance	MoA[a]	Source	Ref[b]
Target: Bacteria and Fungi			
Cinnamaldehyde	Unknown	Cassia plants (*Cassia tora* L.)	(Kang *et al.* 2007; Brown 2013)
Diallyl sulphide	Unknown	*Allium* crops (garlic and onion)	(Davis *et al.* 2007)
Kasugamycin	Protein synthesis inhibitor	*Streptomyces kasugaensis*	(Copping and Duke 2007)
Laminarin	Host plant defense induction	*Laminaria digitata*	(Aziz *et al.* 2003)
Natamycin	Chitin synthase inhibitor	*Streptomyces natalensis*	(te Welscher *et al.* 2010)
Oudemansin	Inhibitor of mitochondrial electron transport (complex III)	*Oudemansiella mucida*	(Akita 2009)
Oxytetracycline	Protein synthesis inhibitor	*Streptomyces rimosus*	(Streltso *et al.* 1974)
Polyoxins	Inhibitor of chitin synthase	*Streptomyces cacaoi*	(Cohen 1993; Zhang and Miller 1999)
Polyoxorims	Chitin synthase inhibitor	*Streptomyces cacaoi*	(Isono and Suzuki 1979)
Rhamnolipid	Cell lysis	*Pseudomonas aeruginosa*	(Sotirova *et al.* 2008)
Validamycin	Trehalase and inositol-biosynthesis inhibitor	*Streptomyces hygroscopicus*	(Kameda *et al.* 1987)
Target: Weeds			
Acetic acid	Unknown	Vinegar	(Reynolds 1974; Rahmat *et al.* 2014)
Bialaphos	Glutamine synthetase inhibitors	*Streptomyces* species	(Duke *et al.* 2000; Duke and Dayan 2011)
Leptospermone	Bleaching: p-hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitor	*Leptospermum scoparium*	(Dayan *et al.* 2011)
D-Limonene	Removing the waxy cuticular layer	*Anethum graveolens* L.	(Dayan and Duke 2010; Shrestha *et al.* 2012)
Pelargonic acid	Unknown	Many plants (including food plants)	(Cantrell *et al.* 2012)
Sorgoleone	Inhibition of photosynthesis at photosystem II	*Sorghum bicolor* L. Moench	(Czarnota *et al.* 2001)
Tabtoxin-β-lactam	Glutamine synthetase inhibitors	*Pseudomonas syringae*	(Duke *et al.* 2000; Duke and Dayan 2011)
a: Classification according to FRAC (Fungicide Resistance Action Committee) for bacteria and fungi (FRAC 2015), and HRAC (Herbicide Resistance Action Committee) for weeds (HRAC 2015) or proposed by independent sources/research works. b: Also references (Dubey 2010; López and Fernández-Bolaños 2011).			

Table 2. Examples of Natural Chemical Compounds with Activity against Insects, Mammals, and Birds

Active Substance	MoA[a]	Source	Ref[b]
Target: Insect			
Azadirachtin	Unknown	*Azadirachta indica*	(López *et al.* 2005)
Capsaicin	Nervous system dysfunction	*Capsicum spp.*	(USEPA 1992; Copping 2001)
Carvone	Repellent	*Mentha spicata* var. *crispa* (Lamiaceae) *Carum carvi* L. (Apiaceae)	(López and Pascual-Villalobos 2010)
Diallyl sulphide	Repellent	*Allium* crops (garlic and onion)	(USEPA 2010a)
Estragole	Repellent / antiaggregant	Estragon and conifer trees	(USEPA 2001; USEPA 2010b)
p-Menthane-3,8-diol	Repellent	*Corymbia citriodora*	(Elmhalli *et al.* 2009)
Nicotine	Nicotinic acetylcholine receptor (nAChR) agonists	*Nicotiana tabacum* L.	(Shi *et al.* 2006)
Pyrethrin I and II	Sodium channel modulators	*Chrysanthemum cinerariaefolium* and *Chrysanthemum coccineum*	(Casida 2011)
Rotenone	Mitochondrial complex I electron transport inhibitors	Tropical plants in the genus *Derris*, *Lonchocarpus* or *Tephrosia*	(Chauvin *et al.* 2001)
Spinosyns	Nicotinic acetylcholine receptor (nAChR) alloxteric activator	*Saccharopolyspora spinosa* and *Saccharopolyspora pogona*	(Kirst *et al.* 1991; Lewer *et al.* 2009)
Target: Mammals and Birds			
9,10-Anthraquinone	Repellent	Plant tannin extracts	(Schrader *et al.* 2003; Cantrell *et al.* 2012)
Capsaicin	Repellent	*Capsicum spp.*	(USEPA 1992)
Diallyl sulphide	Repellent	*Allium* crops (garlic and onion)	(USEPA 2010a)
Piperine	Repellent	*Piper nigrum*	(Ghosh 2000)
a: Classification according to IRAC (Insecticide Resistance Action Committee) for insects (IRAC 2015) or proposed by independent sources/research works. b: Also references (Dubey 2010; López and Fernández-Bolaños 2011).			

The continued use of a pesticide can result in a heritable change in the sensitivity of the target pest population, which leads to the failure of the product to achieve the expected level of control (resistance). In most cases, it also confers cross-resistance to other chemically related compounds that have a common target site, *i.e.*, they share a common MoA. Successful pesticide management prevents the formation of resistances or re-introduces susceptibility in resistant pest populations (IRAC 2015).

Crude preparations (*e.g.*, essential oils, mixtures of fatty acids, and extracts) often present problems related to the combined effect of the biological properties of their constituents, which are unveiled only after their separation (Asili *et al.* 2009). Although crude or partly purified preparations are used in practice, this unsuitable use is changing

because of the variation in composition of the natural sources. Modern legislative frameworks encourage the use of pure, or at least enriched and stabilized products. Chemical modifications are the key to developing new biopesticides from these natural products within the legal framework of every country.

The Role of Chemistry in Biopesticide Development

As mentioned previously, plants are great sources of natural active substances (Domingues *et al.* 2011; Santos *et al.* 2013). Many of these substances contain plant protection activity (Nathan *et al.* 2007), which has facilitated their industrial/commercial exploitation. First, active substances must be isolated and characterized, and their biological activity demonstrated. Active ingredients with crop protection properties should be extracted in a form suitable for commercial formulations, but extraction usually poses many technical problems. Additionally, the development of pesticides should be supported by numerous bioassays to improve the effectiveness of the extracts. Notably, the interest in natural products with phytosanitary activity is due to their innovative molecular structures, not their natural character (Ujváry 2010). In fact, all pesticides are considered unnatural when the working concentration exceeds the concentration in their natural state.

In general, there are three approaches to the development of new biopesticides: a) crude extracts (*e.g.*, from lignocellulosic materials); b) chemical synthesis; and c) computational chemistry-aided pesticide design. Approaches b) and c) are mixed solutions that improve a biopesticide once it has been identified.
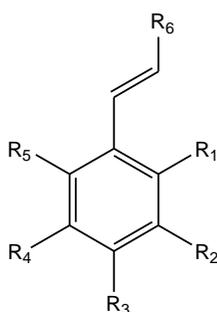
Extraction and purification: plants as sources of active ingredients

New active substances derived from lignocellulosic materials are usually obtained in vegetable extracts produced *via* purification protocols and organic solvents. Polar fractions are extracted with methanol:water mixtures, which enables high gravimetric yields but fairly non-selective and complex extracts. Solvents with a low polarity, such as dichloromethane, are chosen because they are fairly specific to lipophilic extractives (Shen and Shao 2006). However, purification of these fractions requires more selective and environmentally friendly extraction techniques, such as supercritical fluid extraction using carbon dioxide, which is a widely used and sustainable process (Santos *et al.* 2012). The organic fraction is collected, concentrated, and fractionated or purified using column chromatography, and active substances can be identified and quantified on chromatographic systems coupled to detection systems such as mass spectrometry (Qi *et al.* 2008; Jalali *et al.* 2012), or nuclear magnetic resonance techniques when the active substance is isolated in a very pure form (Asili *et al.* 2009).
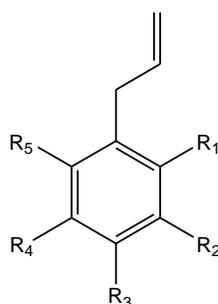
Chemical synthesis of natural active substances

Partial or total chemical synthesis is usually more complex. The synthesis of the compound skeleton, the introduction of functional groups at certain positions, and the stereochemistry of the molecules are all major challenges. However, it is relatively simple to generate many similar chemical structures, using either chemical or biochemical pathways. Thus, the MoA of these compounds can be studied using an approximation of the structure-activity relationship (SAR) (Tewary *et al.* 2006; Bhardwaj *et al.* 2010; Kirst 2010; Li *et al.* 2013). This term describes a rapidly growing field of research that studies relationships between molecular structure (*e.g.*, available hydroxyl (He *et al.* 1997) or polymerization degree (Frackenpohl *et al.* 2009)) and the physicochemical and biological response. For example, Bhardwaj *et al.* (2010) used this approach to evaluate the larvicidal
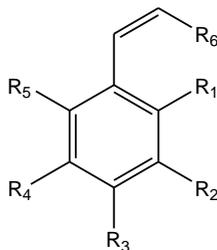
bioresources.com

activity of 18 phenylpropanoids (Fig. 2), including phenylpropenoate, phenylpropenal, phenylpropene, and their semisynthetic analogues against the tobacco armyworm, *Spodoptera litura*. The report identified promising structures with insecticidal activity, observing that OCH_2O substituted phenylpropenes possess insecticidal potential against *Spodoptera litura*. Furthermore, they showed that hydrogenation of isomeric mixtures decreases the susceptibility of the C=C bond to air oxidation, which reduces larvicidal activities, and those problems associated with the separation of isomeric mixtures of phenylpropenes. Therefore, the incorporation of dihydrosafrole in certain formulations could increase their biochemical targets. This effect would limit the appearance of resistances and increase potential synergistic effects, which favors dosage reduction.
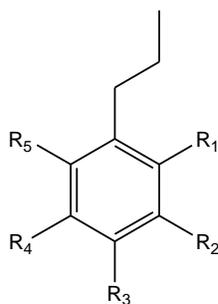

1. R_1, R_2, R_4, R_5 = H; R_3 = OCH_3; R_6 = $COOCH_3$
2. R_1, R_2, R_4, R_5 = H; R_3 = OCH_3; R_6 = CHO
3. R_1, R_2, R_4, R_5 = H; R_3 = OCH_3; R_6 = COOH
4. R_1, R_2, R_4, R_5 = H; R_3 = OCH_3; R_6 = CH_3
5. R_1, R_4, R_5 = H; R_2, R_3 = OCH_3; R_6 = CH_3
6. R_1, R_2, R_5 = H; R_3 & R_4 = OCH_2O; R_6 = CH_3
7. R_1, R_4, R_5 = H; R_2 = OCH_3; R_3 = OH; R_6 = CH_3
8. R_1, R_4 = H; R_2, R_3, R_5 = OCH_3; R_6 = CH_3

10. R_1, R_2, R_4, R_5 = H; R_3 = OCH_3
11. R_1, R_4, R_5 = H; R_2, R_3 = OCH_3
12. R_1, R_2, R_5 = H; R_3 & R_4 = OCH_2O
13. R_1, R_4, R_5 = H; R_2 = OCH_3; R_3 = OH
14. R_1, R_4, R_5 = H; R_2 = OCH_3; R_3 = $OCOCH_3$

9. R_1, R_4 = H; R_2, R_3, R_5 = OCH_3, R_6 = CH_3

15. R_1, R_2, R_4, R_5 = H; R_3 = OCH_3
16. R_1, R_2, R_5 = H; R_3 & R_4 = OCH_2O
17. R_1, R_4, R_5 = H; R_2 = OCH_3; R_3 = OH
18. R_1, R_4 = H; R_2, R_3, R_5 = OCH_3

Fig. 2. Phenylpropanoids (Bhardwaj *et al.* 2010)

Tewary *et al.* (2006) carried out a similar work with *Aphis craccivora*, and they observed that the position of double bonds in the C_6-C_3 system is important because it influences mitochondrial respiration; increasing the activity of phenylpropanoids by non-polar substitutes such as methoxy groups enhances its penetration through the body cuticle and diffusion. In the spinosyn family, over 1000 analogs have been obtained and tested (Salgado and Sparks 2005), and their SARs have been evaluated (Kirst 2010).

SAR allows a qualitative comparison of similar chemical structures and is based on the knowledge that these substances may have similar biological activity. The potential toxic effects of unknown compounds with related chemical structures are predicted using this approach. Understanding how changes in chemical structure affect the magnitude and type of biological effect is also important in SAR studies.

Computational chemistry

According to SAR analysis, the response produced by a substance depends on its chemical structure. Nevertheless, chemical structures cannot be dealt with directly using SAR methodology. Instead, various kinds of molecular descriptors are used, such as fragments (*e.g*., bonds of various types or counts of atoms), geometrical (*e.g*., molecular surface area and volume), physico-chemical (*e.g*., molar refraction), and topological (*e.g*., molecular connectivity and symmetry) (Hansen 2004). Quantitative structure-activity relationship (QSAR) is a statistical tool in which quantitative data concerning specific compounds, such as toxicity, are correlated with several structural parameters of these compounds, such as ionization potential, octanol-water partition coefficient, aqueous solubility, dipole moment, *etc*. (Hansen 2004). The suitability of the QSAR results is highly dependent on the amount and quality of the starting data (input parameters), the descriptors selected (mathematical response functions), and the applied model (Sevilla-Morán *et al.* 2015). QSAR studies employ mainly empirical descriptors, but they can include quantum chemistry, which provides well-defined theoretical descriptors that encode physical-chemical information. These theoretical descriptors can be used in the design of a training set in QSAR studies, and quantum chemistry offers a more accurate and exhaustive description of electronic effects than empirical methods (Cartier and Rivail 1987). The use of quantum chemical descriptors in the phytosanitary industry to develop QSAR models is rare, but there are many methods available for the construction of mathematical models that relate biological properties to chemical structures. Some of the more common techniques used are multiple linear regression (MLR), partial least squares (PLS), principal component regression (PCR), artificial neural networks (ANNs), and discriminant analysis (Livingstone 2004). Currently, QSARs show a broad scope that also allows for risk assessment of chemicals (Cronin 2004; Hansen 2004; Benfenati *et al.* 2007). However, it should be noted that QSARs are estimation methods. Therefore, they should be used as a complementary tool, assisting data evaluation and contributing to the final decision. The exact value of a parameter or property can only be established by experimental studies (Cronin 2004; Hansen 2004; Benfenati *et al.* 2007). To validate QSAR models, measured and predicted values should be compared. Another limitation of QSARs is that relationships can only be established for compounds with a common toxicity mode (Hansen 2004).

China, one of the largest agricultural producers (World Bank 2015), has included the QSAR strategy within the Chinese 973 Green Pesticide Research Program (Qian *et al.* 2010) to discover new green crop protection chemicals with novel MoA, high selectivity to pest species, and low environmental, animal, and human risk. Recently, the role of QSAR in herbicide discovery was revised, such that the first QSAR works were mainly related to the elucidation of novel MoA (Clark 2011). However, the current literature contains techniques for generating QSAR models such as docking or comparative molecular field analysis (CoMFA) to model interactions between individual ligands and the proteins that bind them (Fratev *et al.* 2007). These techniques require regression models such as the PLS method (Tong *et al.* 2004). A Web of Science (2015) search of papers

published in the last decade using the keywords "pesticide QSAR" returned only 237 hits. Thus, biopesticide research combining the SAR and QSAR methods improves biopesticide activity (Sparks *et al.* 2001), and the emerging interest in sustainable pesticides predicts great potential in moving forward in the development of new tools to improve agricultural practices and combat new resistances.

Biopesticides as a Tool in the Integrated Pest Management Practices in the European Union

The disadvantages of using pesticides include secondary infestation, development of resistances, damage to non-target species, water pollution, the presence of residues, and human and animal health risks (Xi *et al.* 2014). However, pesticide use will continue to be advantageous while the main priorities of any society is the feeding of its population and disease control, at least until the appearance of a real alternative to pesticides. However, developed countries demand decreasing pesticides to reduce their residues in food and the environment. To address these problems, the rational use of pesticides must be combined with other practices and control measures in the form of integrated pest management (IPM), taking into account the current developments.

The European Union has enacted the registration of low-risk substances through regulations that provide incentives to biopesticide use (European Commission 2009b) and the required implementation of IPM practices (European Commission 2009a). Both Regulation (EC) No. 1107/2009 (European Commission 2009b) and Directive 2009/128/EC for the sustainable use of pesticides (European Commission 2009a) are crucial elements within the actual European Union thematic strategy on the sustainable use of pesticides (European Commission 2006a; European Commission 2006b). The former regulates the approval of active substances and pesticides, while the latter provides references on how to manage field to reduce the use of pesticides and prevent potential environmental contamination. The fundamental objectives of the European Union thematic strategy and Directive 2009/128/EC (European Commission 2009a) are included in Table 3. It is intended to reduce the impact of pesticides on living organisms and the environment. The goal of this strategy is to achieve more effective treatments, which in turn allows for lower doses. The general principles of IPM are described in Annex III of the Directive 2009/128/EC (European Commission 2009a) and are outlined in Table 4 for their implementation the European Union thematic strategy. These objectives attempt to satisfy the full meaning of the IPM highlighted above.

Table 3. Main Objectives of the Thematic Strategy and Directive 2009/128/EC (European Commission 2006a; 2006b; 2009a)

Objective	Typical Action
Reduce impact of pesticides	Promote IPM plans at national level
Coordinate strategies / Continuous improvement	Exchange of information
Check effectiveness of strategy followed	Measuring risk reduction
Avoid wrong use of pesticides	Improve user training
Limit contamination and max. efficacy treatments	Improve application equipment

Within a background that encourages the development of less harmful substances and alternative pest control techniques, biopesticides may be key to the implementation of

IPM practices. This might be achieved by replacing or combining traditional pesticides with biopesticides or by using biopesticides as a basis for the synthesis of new pesticides. Figure 1 shows how the use of the biopesticide term, together with that of IPM practices, is increasing. However, the future state of biopesticides within the European legislative framework will not be easily established because natural products and biologically derived pest management agents differ notably from conventional pesticides in their MoA, chemistry, and complexity. Such differences make them difficult to categorize in terms of relative risk, and this situation generates regulatory barriers to biopesticide commercialization.

Table 4. General Principles of Integrated Pest Management (European Commission 2009a)

Principles	Methods/Tools
Prevent, suppress, and monitor harmful organisms	Suitable agricultural practices
Measures based on monitoring	Robust and scientifically sound threshold values
Non-chemical methods are preferred	Biological, physical, and other methods
Specific pesticides for the target	-
Use pesticides to necessary levels	Reduced doses and frequency
Apply anti-resistance strategies	Pesticides with diverse MoA
Check the success of the measures	Records on use and monitoring

Case Analysis of Biopesticide Authorizations in the European Union

The progression of European legislation on pesticides has been discussed (Villaverde *et al.* 2014). Hence, this section explains the case of several biopesticide active substances that were assessed under the previous European Council Directive 91/414/EEC (European Commission 1991) and the current Regulation (EC) No. 1107/2009 (European Commission 2009b) (Fig. 3).
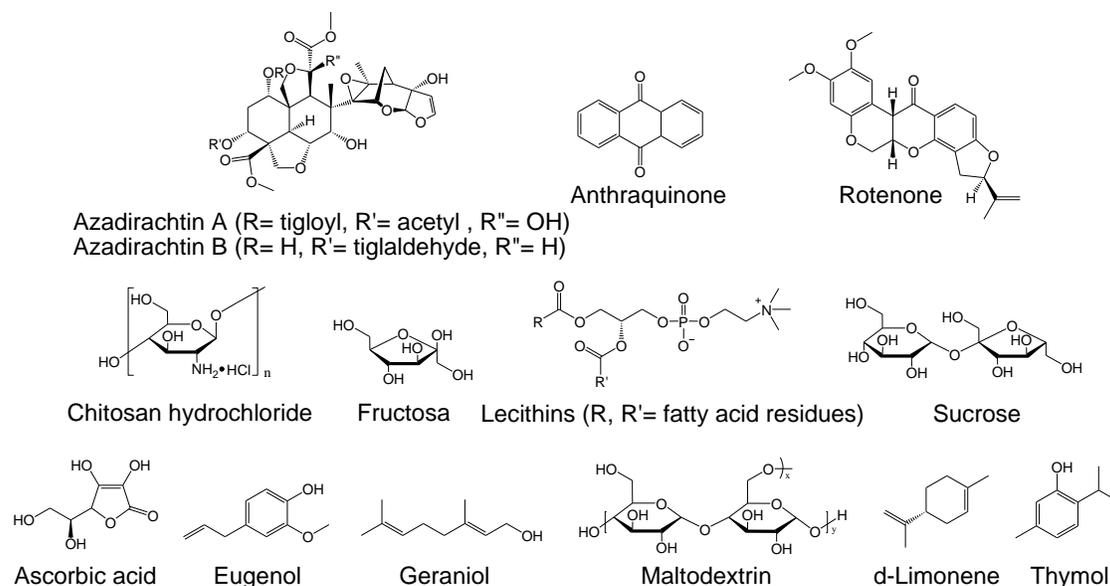

Azadirachtin A (R= tigloyl, R'= acetyl , R"= OH)
Azadirachtin B (R= H, R'= tiglaldehyde, R"= H)

Anthraquinone   Rotenone

Chitosan hydrochloride   Fructosa   Lecithins (R, R'= fatty acid residues)   Sucrose

Ascorbic acid   Eugenol   Geraniol   Maltodextrin   d-Limonene   Thymol

Fig. 3. Some natural active ingredients assessed in Europe

The discussion below includes azadirachtin, rotenone, and anthraquinone whose decision was taken from Council Directive 91/414/EEC (European Commission 1991); and

ascorbic acid, eugenol, geraniol, maltodextrin, orange oil, thymol, chitosan hydrochloride, fructose, lecithins, and sucrose that were transposed into the current Regulation (EC) No. 1107/2009 (European Commission 2009b).

In the fourth stage of the review program of active substances existing in the European Union market before 1993 (European Commission 1991), 295 active substances were considered to be of low risk (European Commission 2015). It could be said that biopesticide active substances were included within this stage; of these, rotenone and anthraquinone are discussed below. Only 112 substances were approved finally. This section also highlights important aspects required for the approval of active substances. Where appropriate, the USEPA assessment is considered.

Before exploring each case, it is important to observe Fig. 4, which shows the stages and deadlines that were followed in the examples evaluated under Regulation (EC) No. 1107/2009 (European Commission 2009b).
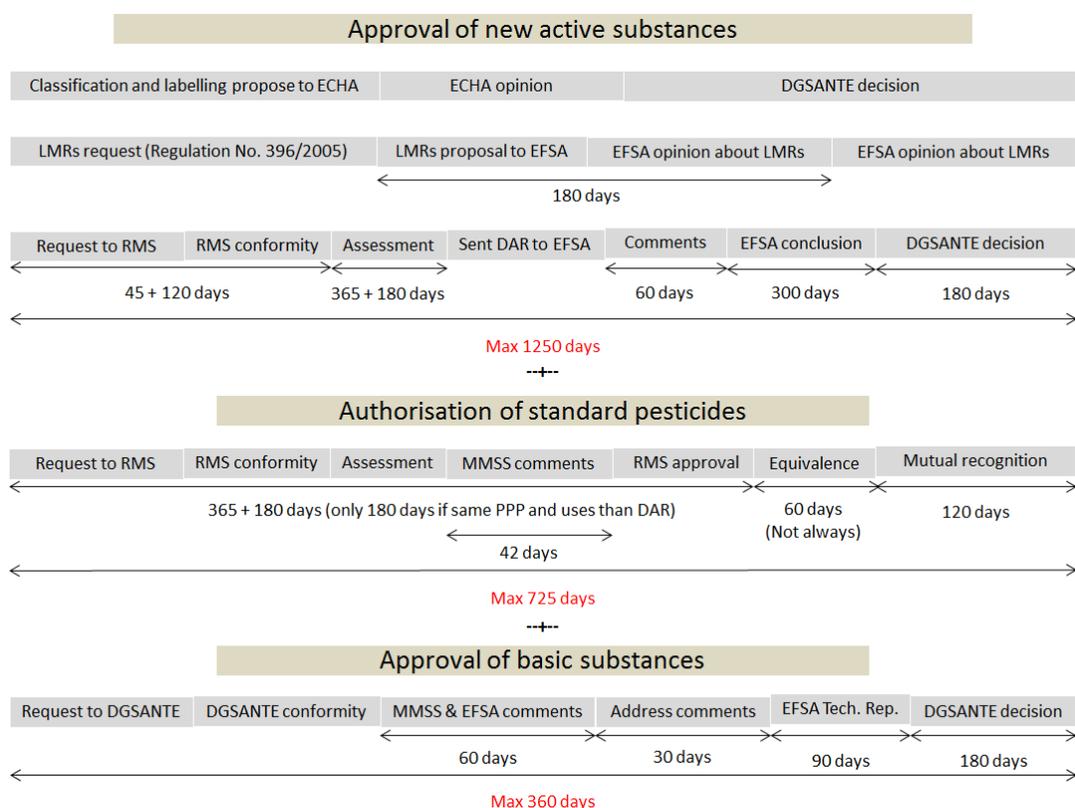

Fig. 4. Timetable for assessment and approval of new active substances under Regulation (EC) No. 1107/2009 (European Commission 2009b), and zonal evaluation and authorization of their commercial formulations. A timetable for approval of basic substances is included for comparison.

Stages and deadlines must be met for both the approval of new active substances and the authorization of pesticides, which accelerates the arrival of new solutions to the market. After the approval of new active substances under Regulation (EC) No. 1107/2009 (European Commission 2009b), which must be performed within 1250 days, the Zonal Rapporteur Member State (Z-RMS) decides whether the pesticide can obtain authorization. Moreover, Z-RMS has 6 months more to request additional information. If the technical equivalence of the active substance must be assessed, then it takes another 60 days. If the

active substance and pesticide were already assessed during the inclusion of the active substance in Annex I, the deadlines are shortened, and Z-RMS must decide within six months. Finally, the short period for the approval of basic substances (360 days) is an excellent opportunity for the approval of low-concern biopesticide active substances.

Azadirachtin, rotenone, and anthraquinone

Although azadirachtin (Fig. 3) was not approved in the first instance, several applicants made a resubmission application together for the inclusion of azadirachtin in Annex I. The resubmission dossier included further data in response to the issues identified in the monograph of azadirachtin. Both evaluation of the additional data and generation of an additional report to the monograph resulted in the recommendation to include azadirachtin in Annex I for its supported uses (European Commission 2011; EFSA 2011).

Rotenone is also an active substance included in the fourth stage programme (Fig. 3). The notifiers for rotenone showed no interest in participating in the programme of work for this active substance. As a consequence, this active substance is not approved. However, the Commission and Member States experts highlighted a need for further use of rotenone. In these circumstances, the period for the withdrawal of authorization for certain essential uses for which there are no efficient alternatives was extended until the year 2011 in Italy, France, and the United Kingdom. However, strict conditions were designed to minimize the possible risks, and this extension was not renewed. Currently, rotenone is not authorized in the European Union (European Commission 2008a). In the USA, most uses have also been voluntarily cancelled by companies (USEPA 2007), maintaining its use as a piscicide. Thus, registration is limited to applications to manage fish populations and getting aquatic invasive species control by assisting native fish and aquatic species towards recovery (USEPA 2007). This example shows how a product is not always removed from the market based only on its toxicological properties, but also due to commercial or economic interests. Azadirachtin may have suffered the same fate. Europe (and rest of the world) faces a difficult approach in that they should use all the means available to safeguard essential health and environmental protection, while still trying to avoid a situation in which many substances are lost because data requirements cannot be fulfilled economically or notifiers are not interested in certain types of substances.

Anthraquinone (Fig. 3) is a bioactive active substance used as a repellent for birds in cereals, and it was also included in the fourth stage programme. It is usually formulated with insecticides and fungicides. During the European examination of this active substance by RMS, Member States, and European Commission, it was concluded that this active substance has harmful effects on human health. The European Commission invited the notifier to submit their comments on the results of the examination of anthraquinone. After consideration of the notifier comments, the identified concerns were not be eliminated. Therefore, under the proposed conditions of use, anthraquinone was not be approved (European Commission 2008b). In contrast, anthraquinone is approved in the USA as a selective mammal and bird repellent. This is a clear example of how the adoption of a substance can also be conditioned by the mode of use, among other factors.

bioresources.com

Ascorbic acid, eugenol, geraniol, maltodextrin, orange oil, and thymol

All substances mentioned in this subsection have been recently approved. Therefore, they were not included in any of the previous review programs. The steps for their approval were consistent with those highlighted in Fig. 4. The greater cooperation between Member States and the more harmonized system achieved in Regulation (EC) No. 1107/2009 (European Commission 2009b) allows the evaluation process to be more standard. However, it is still necessary to work on the harmonization process to achieve a more homogeneous market for pesticides. The major modifications regarding Council Directive 91/414/EEC (European Commission 1991) are those related to the active substances. In this sense, Article 4 and Annex II define approval conditions, including new hazard criteria based on the inherent properties of substances. Moreover, substances may be approved and listed in a separate commission regulation in several categories that comprise basic, low-risk, and standard substances, and candidates for substitution. The former have the best approval and renewal periods, while the latter have greater difficulties in that area. This fact clearly favours the development and use of substances with lower risk. Ascorbic acid, eugenol, geraniol, maltodextrin, orange oil, and thymol (Fig. 3) were classified as standard substances that are approved for up to 10 years with a renewal for 15 years, although they are not substances of special concern.

Chitosan hydrochloride, fructose, lecithins, and sucrose

Recital 18 of the European Regulation (EC) No. 1107/2009 (European Commission 2009b) states that "*specific provisions should ensure that substances which are not predominantly used as pesticides, as far as their risks are acceptable, may also be approved for plant protection use*". In this sense, they should also "*be of value for plant protection, but the economic interest of applying for approval may be limited*". Based on these particular requirements, Article 23 defines a new category of active substances categorized as "basic substances" and fixes the next four criteria for their approval (European Commission 2009b) as: a) not being of concern; b) does not entail endocrine disrupting, neurotoxic or immunotoxic effects; c) not to be used for plant protection, and d) not to be marketed as pesticides. Moreover, those active substances which meet the criteria of a "foodstuff", as established in Article 2 of Regulation (EC) No. 178/2002 (European Commission 2002), are also considered basic substances. Chitosan hydrochloride, fructose, lecithins, and sucrose (Fig. 3) are not used as pesticides, but show some special features that give them the capacity for pest control. These are some of the first basic substances approved recently in Europe (first approval was in June 2014) and constitutes a group of compounds not included in any other legislation. In their application as basic substances, which may be submitted by a Member State or any interested party to the Commission, they were only required to present a small dossier that mainly only included the identity of the substances and their predominant uses outside plant protection, together with the intended uses, classification, and labelling of the substance. The rest of the information (*i.e.*, toxicological effects, residues, and environmental fate and behaviour) was provided through results published in scientific literature, and conclusions were supported by well-known evidences. More detailed requirements can be consulted in working document SANCO/10363/2012 (European Commission 2014), where the European Commission establishes the procedure for application of basic substances, to be approved in compliance with Article 23 of Regulation (EC) No 1107/2009 (European Commission 2009b). Article 28 of Regulation (EC) No 1107/2009 establishes that basic substances do not require additional authorization for their marketing and use as pesticides,

as long as they are formulated only with basic substances (European Commission 2009b). Moreover, basic substances do not need be renewed. However, the European Commission may review their approval at any time, and conditions and restrictions for approval can be requested in agreement with Article 6 of Regulation (EC) No 1107/2009 (European Commission 2009b). Nevertheless, this means a lower cost and faster product approval and registration for substances other than the substances and products commented on previously (Fig. 4). In addition, their approval as basic substances gives them an appropriate legal framework, which ensures them as good future prospects.

Current and Future Trends

Biopesticides should be a cornerstone in the development and implementation of resistance management programs. The development of new biopesticides by agrochemical companies is essential to safeguard their activities. In this sense, it is imperative that funds are provided to support the search for new MoA that remain high in resistance management. The aim of this should be the search of novel biopesticide active substances with different MoA involving the inhibition of metabolic processes that also involve a low risk of resistance developing. However, it is unrealistic to think that biopesticide use can totally replace conventional chemical pesticides, except in organic food production.

Agrochemical companies might be changing their market and R&D strategies because of evolution of resistances to conventional chemical pesticides, and because of modern legislations more protective of human, animal, and environmental health (*e.g.* Regulation (EC) No. 1107/2009) (European Commission 2009b). Legislation should surely involve the adoption of future specific policies for new or modified natural substances with plant protection activity.

Resistance risk analysis is a key requirement, as efficacy demonstration of pesticide active substances and their formulations are mandatory according to Regulation No. 283/2013 (European Commission 2013a) and Regulation No. 284/2013 (European Commission 2013b), respectively. The European and Mediterranean Plant Protection Organization (EPPO) considers the "resistance risk analysis" (EPPO© 2015) to be a two-stage process composed of "resistance risk assessment" and "resistance risk management". The former examines the probability of resistance development, while the latter recommends strategies to avoid the development of resistance. The resistance risk assessment requires examination of the inherent risk (associated with the MoA of the product and the pest) and the agronomic risk (influenced by the crop, the geographic area, and the use pattern). The resistance risk assessment can be influenced by multiple factors (*e.g.* identity and family of the active substance, both MoA and mechanism of resistances, cross-resistance, behaviour of the resistant strains, consequences of a limitless use, and level of resistance risk) and they should be analyzed case by case. After the resistance risk assessment stage, resistance risk management should be performed if the risk in unrestricted use is unacceptable (high inherent and agronomic risks). Otherwise (low inherent and agronomic risks, or even high inherent risk and low agronomic risk), the baseline sensitivity should be established directly through monitoring before the introduction of the active substance. Finally, a dossier for registration should be displayed and efficacy of the pesticide should be monitored. Therefore, resistance risk analysis is a complex issue, and the basis for risk assessment, which requires a clear willingness on the stakeholders to co-operate in order to maintain a sustainable, viable, and safe agricultural environment within the European Union.

Three chemical approaches are essential to discover or efficiently develop new biopesticides with novel modes and mechanisms of action: extraction from natural sources, chemical synthesis, and computational chemistry. This integrated process and rational design involves indirect biological and toxicological studies. All these studies should be addressed together using proper scientific method and research protocols to allow suitable results. Synthesis of pesticides is mainly based upon obtaining a basic structure with a certain function, and the growth of the rest of the molecule around that function. SAR and QSAR models can be used to predict the physicochemical and biological properties, and the environmental fate of pesticides and their transformation products. Both fulfill recital 11 of the European Regulation (EC) No. 1107/2009, which states that "*the development of non-animal test methods should be promoted in order to produce safety data relevant to humans and to replace animal studies currently in use*" (European Commission 2009b).

On the other hand, the full development of IPM practices is crucial in order to favour biopesticide usage. The ideal case would be that conventional pesticides will only be used to minimize the evolution of resistance in target pest populations, in favour of widespread biopesticides and other friendly environmental technologies. The well-consolidated conventional pesticides applied on a calendar basis with suitable results are difficult to change to an alternative IPM practice using biopesticides. However, recent published works focused on the IPM programs show how correcting pest management practices can reduce pesticide use without negative consequences (Pretty 2008; Sharma *et al.* 2015). Therefore, solutions should be enacted to remove unfounded fears regarding a fall in crop yield, even if it does, when this means a dramatic drop in the use of pesticides. Crop protection strategies should also be developed in order to avoid risks on human, animal, and environmental health.

CONCLUSIONS

1. Due to nature being an almost unlimited source of bioactive natural products, and the great progress in the structural isolation and characterization of these compounds, the current model of relying almost exclusively on synthetic chemicals as a source of pesticides is beginning to change. This fact, together with new European legislation for the registration of plant protection products encouraging the development of less harmful substances, is causing natural products to begin to replace traditional pesticides, or provide the basis for the synthesis of new ones.

2. Extraction from natural sources, chemical synthesis, and use of computational chemistry (*i.e.*, SARs and QSARs) are the current main approaches in the discovery and development of biopesticides.

3. There is no doubt that biopesticides represent an attractive technology from the standpoint of improved European IPM programs. Moreover, biopesticides, as high value chemicals obtained from lignocellulosic materials, can be considered to be of high interest within the biorefinery concept in order to achieve an integrated exploitation of residues from agroforest industries.

4. It is important to be aware that biopesticides are not inoffensive, and that they fall under the same state and regulations as synthetic pesticides. Therefore, they must be used

only as indicated on the label, which provides critical information about how to safely handle and use pesticides.

REFERENCES CITED

Akita, H. (2009). "Recent advances in the syntheses of biologically active natural products using biocatalyst," *Heterocycles* 78(7), 1667-1713. DOI: 10.3987/rev-09-652

Aliferis, K. A., and Jabaji, S. (2011). "Metabolomics - A robust bioanalytical approach for the discovery of the modes-of-action of pesticides: A review," *Pest. Biochem. Physiol.* 100(2), 105-117. DOI: 10.1016/j.pestbp.2011.03.004

Asili, J., Sahebkar, A., Bazzaz, B. S. F., Sharifi, S., and Iranshahi, M. (2009). "Identification of essential oil components of *Ferula badrakema* fruits by GC-MS and ^{13}C-NMR methods and evaluation of its antimicrobial activity," *J. Essent. Oil Bear. Plants.* 12(1), 7-15.

Aziz, A., Poinssot, B., Daire, X., Adrian, M., Bezier, A., Lambert, B., Joubert, J. M., and Pugin, A. (2003). "Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*," *Mol. Plant-Microbe Interact.* 16(12), 1118-1128. DOI: 10.1094/mpmi.2003.16.12.1118

Benfenati, E., Clook, M., Fryday, S., and Hart, A. (2007). "QSARs for regulatory purposes: The case for pesticide authorization," in: *Quantitative Structure-Activity Relationships (QSAR) for Pesticide Regulatory Purposes*, Benfenati, E., and Benfenati, E. (eds.), Elsevier Science, Amsterdam, Netherlands, pp.1-57. DOI: 10.1016/B978-044452710-3/50003-3

Bhardwaj, A., Tewary, D. K., Kumar, R., Kumar, V., Sinha, A. K., and Shanker, A. (2010). "Larvicidal and structure-activity studies of natural phenylpropanoids and their semisynthetic derivatives against the tobacco armyworm *Spodoptera litura* (FAB.) (*Lepidoptera*: *Noctuidae*)," *Chem. Biodivers.* 7(1), 168-177.

Brown, A. E. (2013). *Mode of Action of Pesticides and Related Pest Control Chemicals for Production Agriculture, Ornamentals and Turf*, University of Maryland, College Park, MD, USA.

Cantrell, C. L., Dayan, F. E., and Duke, S. O. (2012). "Natural products as sources for new pesticides," *J. Nat. Prod.* 75(6), 1231-1242. DOI: 10.1021/np300024u

Cartier, A., and Rivail, J. L. (1987). "Electronic descriptors in quantitative structure-activity relationships," *Chemom. Intell. Lab. Syst.* 1(4), 335-347. DOI:10.1016/0169-7439(87)80039-4

Casida, J. E. (2009). "Pest Toxicology: The primary mechanisms of pesticide action," *Chem. Res. Toxicol.* 22(4), 609-619. DOI: 10.1021/tx8004949

Casida, J. E. (2011). "Curious about pesticide action," *J. Agric. Food Chem.* 59(7), 2762-2769. DOI: 10.1021/jf102111s

Chandler, D., Bailey, A. S., Tatchell, G. M., Davidson, G., Greaves, J., and Grant, W. P. (2012). "The development, regulation and use of biopesticides for integrated pest management," *Philos. Trans. R. Soc. B-Biol. Sci.* 366(1573), 1987-1998. DOI: 10.1098/rstb.2010.0390

Chauvin, C., De Oliveira, F., Ronot, X., Mousseau, M., Leverve, X., and Fontaine, E. (2001). "Rotenone inhibits the mitochondrial permeability transition-induced cell

death in U937 and KB cells," *J. Biol. Chem.* 276(44), 41394-41398. DOI: 10.1074/jbc.M106417200

Clark, R. D. (2011). "A perspective on the role of quantitative structure-activity and structure-property relationships in herbicide discovery," *Pest Manag. Sci.* 68(4), 513-518. DOI: 10.1002/ps.3256

Cohen, E. (1993). "Chitin synthesis and degradation as targets for pesticide action," *Arch. Insect Biochem. Physiol.* 22(1-2), 245-261. DOI: 10.1002/arch.940220118

Copping, L. G. (2001). *The BioPesticide Manual* (2nd Ed.), British Crop Protection Council, Farnham, UK.

Copping, L. G., and Duke, S. O. (2007). "Natural products that have been used commercially as crop protection agents," *Pest Manag. Sci.* 63(6), 524-554. DOI: 10.1002/ps.1378

Cronin, M. T. D. (2004). "The use by governmental regulatory agencies of quantitative structure-activity relationships and expert systems to predict toxicity," in: *Predicting Chemical Toxicity and Fate*, Cronin, M. T. D., and Livingstone, D. J. (eds.), CRC Press, Boca Raton, FL, USA, pp. 413-427. DOI: 10.1201/9780203642627.ch19

Czarnota, M. A., Paul, R. N., Dayan, F. E., Nimbal, C. I., and Weston, L. A. (2001). "Mode of action, localization of production, chemical nature, and activity of sorgoleone: A potent PSII inhibitor in *Sorghum spp*. root exudates," *Weed Technol.* 15(4), 813-825. DOI: 10.1614/0890-037x(2001)015[0813:moalop]2.0.co;2

Davis, R. M., Hao, J. J., Romberg, M. K., Nunez, J. J., and Smith, R. F. (2007). "Efficacy of germination stimulants of sclerotia of *Sclerotium cepivorum* for management of white rot of garlic," *Plant Dis.* 91(2), 204-208. DOI: 10.1094/pdis-91-2-0204

Dayan, F. E., Cantrell, C. L., and Duke, S. O. (2009). "Natural products in crop protection," *Bioorg. Med. Chem.* 17(12), 4022-4034. DOI: 10.1016/j.bmc.2009.01.046

Dayan, F. E., and Duke, S. O. (2010). "Natural products for weed management in organic farming in the USA," *Outlooks Pest Manag.* 21(4), 156-160. DOI: 10.1564/21aug02

Dayan, F. E., Howell, J., Marais, J. P., Ferreira, D., and Koivunen, M. (2011). "Manuka oil, a natural herbicide with preemergence activity," *Weed Sci.* 59(4), 464-469. DOI: 10.1614/ws-d-11-00043.1

Domingues, R. M. A., Patinha, D. J. S., Sousa, G. D. A., Villaverde, J. J., Silva, C. M., Freire, C. S. R., Silvestre, A. J. D., and Neto, C. P. (2011). "*Eucalyptus* biomass residues from agro-forest and pulping industries as sources of high-value triterpenic compounds," *Cell. Chem. Technol.* 45(7-8), 475-481.

Dubey, N. K. (2010). *Natural Products in Plant Pest Management*, CAB International, Wallingford, UK.

Duke, S. O., and Dayan, F. E. (2011). "Modes of action of microbially produced phytotoxins," *Toxins* 3(8), 1038-1064. DOI: 10.3390/toxins3081038

Duke, S. O., Dayan, F. E., Romagni, J. G., and Rimando, A. M. (2000). "Natural products as sources of herbicides: Current status and future trends," *Weed Res.* 40(1), 99-111. DOI: 10.1046/j.1365-3180.2000.00161.x

European Commission (EC) Directive 91/414/EEC. (1991). "Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC)," European Union, Brussels, Belgium.

European Commission (EC) Regulation 178/2002. (2002). "Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European

Food Safety Authority and laying down procedures in matters of food safety," European Union, Brussels, Belgium.

European Commission (EC) Communication COM(2006) 372 final. (2006a). "Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions. A Thematic Strategy on the Sustainable Use of Pesticides," European Union, Brussels, Belgium.

European Commission (EC) Working Document SEC(2006) 895. (2006b). "Commission Staff Working Document Accompanying the Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions. A Thematic Strategy on the Sustainable Use of Pesticides. Technical Annex," European Union, Brussels, Belgium.

European Commission (EC) Decision 2008/317/EC. (2008a). "Commission Decision of 10 April 2008 concerning the non-inclusion of rotenone, extract from equisetum and chinin-hydrochlorid in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing these substances (2008/317/EC)," European Union, Brussels, Belgium.

European Commission (EC) Decision 2008/986/EC. (2008b). "Commission decision of 15 December 2008 concerning the non-inclusion of antraquinone in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance (2008/986/EC)," European Union, Brussels, Belgium.

European Commission (EC) Directive 2009/128/EC. (2009a). "Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides," European Union, Brussels, Belgium.

European Commission (EC) Regulation 1107/2009. (2009b). "Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC," European Union, Brussels, Belgium.

European Commission (EC) Review report SANCO/10311/2011 final. (2011). "Review report for the active substance azadirachtin finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 11 March 2011 in view of the inclusion of azadirachtin to Annex I of Directive 91/414/EEC," European Union, Brussels, Belgium.

European Commission (EC) Regulation 283/2013. (2013a). "Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market," European Union, Brussels, Belgium.

European Commission (EC) Regulation 284/2013. (2013b). "Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market," European Union, Brussels, Belgium.

European Commission (EC) Working Document SANCO/10363/2012 rev.9. (2014). Working document on the procedure for application of basic substances to be approved in compliance with Article 23 of Regulation (EC) No 1107/2009," European Union, Brussels, Belgium.

European Commission (EC) Pesticides Database. (2015). European Commission Health & Consumers Directorate-General, (http://ec.europa.eu).

European Food Safety Authority (EFSA). (2011). "Conclusion on the peer review of the pesticide risk assessment of the active substance azadirachtin," *EFSA J.* 9(3), 1858-1934. DOI:10.2903/j.efsa.2011.1858

Elmhalli, F. H., Palsson, K., Orberg, J., and Jaenson, T. G. T. (2009). "Acaricidal effects of *Corymbia citriodora* oil containing para-menthane-3,8-diol against nymphs of *Ixodes ricinus* (*Acari*: *Ixodidae*)," *Exp. Appl. Acarol.* 48(3), 251-262. DOI: 10.1007/s10493-009-9236-4

Ellgehausen, H., Guth, J. A., and Esser, H. O. (1980). "Factors determining the bioaccumulation potential of pesticides in the individual compartments of aquatic food-chains," *Ecotox. Environ. Safe.* 4(2), 134-157. DOI: 10.1016/0147-6513(80)90015-9

EPPO©. (2015). "EPPO Standards - Efficacy evaluation of plant protection products - PP 1/213 (4) Resistance risk analysis," *EPPO Bulletin* 0(0), 1-17. DOI: 10.1111/epp.12246

FRAC. (2015). *FRAC Code List© 2015: Fungicides sorted by mode of action*, Fungicide Resistance Action Committee, (http://www.frac.info), Accessed Nov. 2015.

Frackenpohl, J., Adelt, I., Antonicek, H., Arnold, C., Behrmann, P., Blaha, N., Bohmer, J., Gutbrod, O., Hanke, R., Hohmann, S., *et al*. (2009). "Insecticidal heterolignans-Tubuline polymerization inhibitors with activity against chewing pests," *Bioorg. Med. Chem.* 17(12), 4160-4184. DOI: 10.1016/j.bmc.2009.01.042

Fratev, F., Lo Piparo, E., Benfenati, E., and Mihaylova, E. (2007). "Toxicity study of allelochemical-like pesticides by a combination of 3D-QSAR, docking, Local Binding Energy (LBE) and GRID approaches," *SAR QSAR Environ. Res.* 18(7-8), 675-692. DOI: 10.1080/10629360701428920

Geyter, E., Lambert, E., Geelen, D., and Smagghe, G. (2007). "Novel advances with plant saponins as natural insecticides to control pest insects," *Pest Technol.* 1(2), 96-105.

Ghosh, G. K. (2000). *Biopesticide & Integrated Pest Management*, APH Publishing Corporation, New Delhi.

Hansen, O. C. (2004). *Pesticides Research No. 94: Quantitative Structure-Activity Relationships (QSAR) and Pesticides*, Danish Environmental Protection Agency, Copenhagen, Denmark.

He, K., Zeng, L., Ye, Q., Shi, G. E., Oberlies, N. H., Zhao, G. X., Njoku, J., and McLaughlin, J. L. (1997). "Comparative SAR evaluations of Annonaceous acetogenins for pesticidal activity," *Pestic. Sci.* 49(4), 372-378. DOI: 10.1002/(sici)1096-9063(199704)49:4<372::aid-ps543>3.0.co;2-k

Howard, L. O. (1935). *La menace des insectes*, Flammarion, Paris, France.

HRAC. (2015). "Classification of herbicides according to site of action," Herbicide Resistance Action Committee, (http://www.hracglobal.com), Accessed Nov. 2015.

Hunt, E. G., and Bischoff, A. I. (1960). "Inimical effects on wildlife of periodic DDD applications to Clear Lake," *Calif. Fish Game* 46, 91-106.

bioresources.com

IRAC. (2015). "IRAC mode of action (MoA) classification," Insecticide Resistance Action Committee, (http://www.irac-online.org), Accessed Nov. 2015.

Isono, K., and Suzuki, S. (1979). "Polyoxins - Pyrimidine nucleoside peptide antibiotics inhibiting fungal cell-wall biosynthesis," *Heterocycles* 13(1), 333-351. DOI: 10.3987/S-1979-01-0333

Jalali, H. T., Petronilho, S., Villaverde, J. J., Coimbra, M. A., Domingues, M. R. M., Ebrahimian, Z. J., Silvestre, A. J. D., and Rocha, S. M. (2012). "Deeper insight into the monoterpenic composition of *Ferula gummosa* oleo-gum-resin from Iran," *Ind. Crop. Prod.* 36(1), 500-507. DOI: 10.1016/j.indcrop.2011.11.001

Kameda, Y., Asano, N., Yamaguchi, T., and Matsui, K. (1987). "Validoxylamines as trehalase inhibitors," *J. Antibiot.* 40(4), 563-565. DOI: 10.7164/antibiotics.40.563

Kang, T. H., Hwang, E. I., Yun, B. S., Park, K. D., Kwon, Y. M., Shin, C. S., and Kim, S. U. (2007). "Inhibition of chitin synthases and antifungal activities by 2'-benzoyloxy-cinnamaldehyde from *Pleuropterus ciliinervis* and its derivatives," *Biol. Pharm. Bull.* 30(3), 598-602. DOI: 10.1248/bpb.30.598

Kato-Noguchi, H., Ota, K., Kujime, H., and Ogawa, M. (2013). "Effects of momilactone on the protein expression in *Arabidopsis* germination," *Weed Biol. Manag.* 13(1), 19-23. DOI: 10.1111/wbm.12005

Kirst, H. A. (2010). "The spinosyn family of insecticides: Realizing the potential of natural products research," *J. Antibiot.* 63(3), 101-111. DOI: 10.1038/ja.2010.5

Kirst, H. A., Michel, K. H., Martin, J. W., Creemer, L. C., Chio, E. H., Yao, R. C., Nakatsukasa, W. M., Boeck, L., Occolowitz, J. L., Paschal, J. W., *et al.* (1991). "A83543A-D, unique fermentation-derived tetracyclic macrolides," *Tetrahedron Lett.* 32(37), 4839-4842. DOI: 10.1016/s0040-4039(00)93474-9

Leduc, R., Unny, T. E., and McBean, E. A. (1987). "Stochastic modeling of the insecticide fenitrothion in water and sediment compartments of a stagnant pond," *Water Resour. Res.* 23(7), 1105-1112. DOI: 10.1029/WR023i007p01105

Lehr, P. (2010). *Biopesticides: The Global Market (Code CHM029C)*, BCC Research LLC., (http://www.bccresearch.com/market-research), Accessed Nov. 2015.

Leng, P., Zhang, Z., Pan, G., and Zhao, M. (2011). "Applications and development trends in biopesticides," *Afr. J. Biotechnol.* 10(86), 19864-19873. DOI: 10.5897/AJBX11.009

Lewer, P., Hahn, D. R., Karr, L. L., Duebelbeis, D. O., Gilbert, J. R., Crouse, G. D., Worden, T., Sparks, T. C., Edwards, P. M. R., and Graupner, P. R. (2009). "Discovery of the butenyl-spinosyn insecticides: Novel macrolides from the new bacterial strain *Saccharopolyspora pogona*," *Bioorg. Med. Chem.* 17(12), 4185-4196. DOI: 10.1016/j.bmc.2009.02.035

Li, M., Liu, C. L., Zhang, J., Wu, Q., Hao, S. L., and Song, Y. Q. (2013). "Design, synthesis and structure-activity relationship of novel insecticidal dichloro-allyloxy-phenol derivatives containing substituted pyrazol-3-ols," *Pest Manag. Sci.* 69(5), 635-641. DOI: 10.1002/ps.3417

Livingstone, D. J. (2004). "Building QSAR models: A practical guide," in: *Predicting Chemical Toxicity and Fate*, M. T. D. Cronin and D. J. Livingstone (eds.), CRC Press, Boca Raton, FL, USA, pp. 151-170. DOI: 10.1201/9780203642627.ch7

López, M. D., and Pascual-Villalobos, M. J. (2010). "Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control," *Ind. Crop. Prod.* 31(2), 284-288. DOI: 10.1016/j.indcrop.2009.11.005

López, O., and Fernández-Bolaños, J. G. (2011). *Green Trends in Insect Control*, RSC Publishing, Cambridge, UK. DOI:10.1039/9781849732901

López, O., Fernández-Bolaños, J. G., and Gil, M. V. (2005). "New trends in pest control: The search for greener insecticides," *Green Chem.* 7(6), 431-442. DOI: 10.1039/b500733j

Market and Markets. (2015). *Global Biopesticides Market Trends & Forecasts (2012 – 2017) (Report Code: CH 1266)*, (www.marketsandmarkets.com), Accessed Nov. 2015.

Marrone, P. G. (2007). "Barriers to adoption of biological control agents and biological pesticides," *CAB Reviews* 2(51), 1-12. DOI: 10.1079/PAVSNNR20072051

Mouches, C., Pasteur, N., Berge, J. B., Hyrien, O., Raymond, M., Desaintvincent, B. R., Desilvestri, M., and Georghiou, G. P. (1986). "Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito," *Science* 233(4765), 778-780. DOI: 10.1126/science.3755546

Nathan, S. S., Choi, M. Y., Paik, C. H., Seo, H. Y., Kim, J. D., and Kang, S. M. (2007). "The toxic effects of neem extract and azadirachtin on the brown planthopper, *Nilaparvata lugens* (Stål) (BPH) (*Homoptera*: *Delphacidae*)," *Chemosphere* 67(1), 80-88. DOI: 10.1016/j.chemosphere.2006.09.045

Pretty, J. (2008). "Agricultural sustainability: Concepts, principles and evidence," *Philos. Trans. R. Soc. B.* 363(1491), 447-465. DOI: 10.1098/rstb.2007.2163

Qi, L. W., Li, J. C. P., Yu, Q. T., Wen, X. D., Wang, Y. X., Li, C. Y., Bao, K. D., Ge, X. X., and Cheng, X. L. (2008). "Qualitative and quantitative analysis of Radix Astragali products by fast high-performance liquid chromatography-diode array detection coupled with time-of-flight mass spectrometry through dynamic adjustment of fragmentor voltage," *J. Chromatogr. A* 1203(1), 27-35. DOI: 10.1016/j.chroma.2008.07.019

Qian, X. H., Lee, P. W., and Cao, S. (2010). "China: Forward to the green pesticides via a basic research program," *J. Agric. Food Chem.* 58(5), 2613-2623. DOI: 10.1021/jf904098w

Rahmat, B., Pangesti, D., Natawijaya, D., and Sufyadi, D. (2014). "Generation of wood-waste vinegar and its effectiveness as a plant growth regulator and pest insect repellent," *BioResources* 9(4), 6350-6360.

Regnault-Roger, C., Philogene, B. J. R., and Vincent, C. (2005). *Biopesticides of Plant Origin*, Intercept Ltd., Wimborne, UK.

Reynolds, A. E. (1974). *The Mode of Action of Acetic Acid on Bacteria*, University Microfilms, Ann Harbor, MI, USA.

Salgado, V. L., and Sparks, T. C. (2005). "The spinosyns: Chemistry, biochemistry, mode of action and resistance," in: *Comprehensive Molecular Insect Science*, Gilbert, L. J., Iatrou, K., and Gill, S. S. (eds.), Elsevier B.V., Oxford, UK, pp. 137-173. DOI:10.1016/B0-44-451924-6/00078-8

Santos, S. A. O., Villaverde, J. J., Silva, C. M., Neto, C. P., and Silvestre, A. J. D. (2012). "Supercritical fluid extraction of phenolic compounds from *Eucalyptus globulus* Labill bark," *J. Supercrit. Fluids* 71, 71-79. DOI: 10.1016/j.supflu.2012.07.004

Santos, S. A. O., Villaverde, J. J., Sousa, A. F., Coelho, J. F. J., Neto, C. P., and Silvestre, A. J. D. (2013). "Phenolic composition and antioxidant activity of industrial cork by-products," *Ind. Crop. Prod.* 47, 262-269. DOI: 10.1016/j.indcrop.2013.03.015

Schrader, K. K., Dhammika Nanayakkara, N. P., Tucker, C. S., Rimando, A. M., Ganzera, M., and Schaneberg, B. T. (2003). "Novel derivatives of 9,10-anthraquinone

are selective algicides against the musty-odor cyanobacterium *Oscillatoria perornata*," *Appl. Environ. Microbiol.* 69(9), 5319-5327. DOI: 10.1128/aem.69.9.5319-5327.2003

Sevilla-Morán, B., Villaverde, J. J., Mateo-Miranda, M. M., Alonso-Prados, J. L., and Sandín-España, P. (*in press*). "Pesticide transformation products as contaminants of water compartments," in: *Environmental Science & Engineering*, Govil, J. N. (ed.), Studium Press LLC, New Delhi, India.

Sharma, R., Peshin, R., Shankar, U., Kaul, V., and Sharma, S. (2015). "Impact evaluation indicators of an Integrated Pest Management program in vegetable crops in the subtropical region of Jammu and Kashmir, India," *Crop Prot.* 67, 191-199. DOI: 10.1016/j.cropro.2014.10.014

Shen, J. C., and Shao, X. G. (2006). "Determination of tobacco alkaloids by gas chromatography-mass spectrometry using cloud point extraction as a preconcentration step," *Anal. Chim. Acta* 561(1-2), 83-87. DOI: 10.1016/j.aca.2006.01.002

Shi, Q. M., Li, C. J., and Zhang, F. S. (2006). "Nicotine synthesis in *Nicotiana tabacum* L. induced by mechanical wounding is regulated by auxin," *J. Exp. Bot.* 57(11), 2899-2907. DOI: 10.1093/jxb/erl051

Shrestha, A., Moretti, M., and Mourad, N. (2012). "Evaluation of thermal implements and organic herbicides for weed control in a nonbearing almond (*Prunus dulcis*) orchard," *Weed Technol.* 26(1), 110-116. DOI: 10.1614/wt-d-11-00083.1

Sotirova, A. V., Spasova, D. I., Galabova, D. N., Karpenko, E., and Shulga, A. (2008). "Rhamnolipid-biosurfactant permeabilizing effects on gram-positive and gram-negative bacterial strains," *Curr. Microbiol.* 56(6), 639-644. DOI: 10.1007/s00284-008-9139-3

Sparks, T. C., Crouse, G. D., and Durst, G. (2001). "Natural products as insecticides: The biology, biochemistry, and quantitative structure-activity relationships of spinosyns and spinosoids," *Pest Manag. Sci.* 57(10), 896-905. DOI: 10.1002/ps.358

Streltso, S. A., Kukhanov, M. K., Krayevsk, A. A., Beljavsk, I. V., Victorov, L. S., Gursky, G. V., Trebogan, A. D., and Gottikh, B. P. (1974). "Binding of oxytetracycline to *Escherichia-coli* ribosomes," *Mol. Biol. Rep.* 1(7), 391-396. DOI: 10.1007/bf00385671

te Welscher, Y. M., Jones, L., van Leeuwen, M. R., Dijksterhuis, J., de Kruijff, B., Eitzen, G., and Breukink, E. (2010). "Natamycin inhibits vacuole fusion at the priming phase via a specific interaction with ergosterol," *Antimicrob. Agents Chemother.* 54(6), 2618-2625. DOI: 10.1128/aac.01794-09

Tewary, D. K., Bhardwaj, A., Sharma, A., Sinha, A. K., and Shanker, A. (2006). "Bioactivity and structure-activity relationship of natural methoxylated phenylpropenes and their derivatives against *Aphis craccivora* Koch (*Hemiptera*: *Aphididae*)," *J. Pest Sci.* 79(4), 209-214. DOI: 10.1007/s10340-006-0135-8

Tong, W. D., Fang, H., Hong, H. X., Xie, Q., Perkins, R., and Sheehan, D. M. (2004). "Receptor-mediated toxicity: QSARs for estrogen receptor binding and priority setting of potential estrogenic endocrine disruptors," in: *Predicting Chemical Toxicity and Fate*, Cronin, M. T. D., and Livingstone, D. J. (eds.), CRC Press, Boca Raton, FL, USA, pp. 285-314. DOI: 10.1201/9780203642627.ch13

Ujváry, I. (2010). "Pest control agents from natural products," in: *Hayes' Handbook of Pesticide Toxicology* (3th ed.), Krieger, R. (ed.), Academic Press, New York, USA, pp.119-229. DOI: 10.1016/B978-0-12-374367-1.00003-3

bioresources.com

USEPA. (1992). "Reregistration eligibility document (RED): Capsaicin," United States Environmental Protection Agency, (http://www.epa.gov), Accessed Nov. 2015.

USEPA. (2001). "4-Allyl anisole. Biopesticides registration action document," United States Environmental Protection Agency, (http://www.epa.gov), Accessed Nov. 2015.

USEPA. (2007). "Reregistration eligibility decision for rotenone (EPA 738-R-07-005)," United States Environmental Protection Agency, (http://www.epa.gov), Accessed Nov. 2015.

USEPA. (2010a). "Garlic oil: Proposed registration review final decision. Case 4007," United States Environmental Protection Agency, (http://www.epa.gov), Accessed Nov. 2015.

USEPA. (2010b). "Registration review: Verbenone and 4-allyl anisole (estragole). Final work plan," United States Environmental Protection Agency, (http://www.epa.gov), Accessed Nov. 2015.

USEPA. (2015). "United States Environmental Protection Agency," United States Environmental Protection Agency, (http://www.epa.gov), Accessed Nov. 2015.

Villaverde, J. J., Sevilla-Morán, B., Sandín-España, P., López-Goti, C., and Alonso-Prados, J. L. (2014). "Biopesticides in the framework of the European Pesticide Regulation (EC) No. 1107/2009," *Pest Manag. Sci.* 70(1), 2-5. DOI: 10.1002/ps.3663

Web of Science. (2015). ISI Web of Knowledge. Thomson Reuters, (https://apps.webofknowledge.com), Accessed Nov. 2015.

World Bank. (2015). *China: Innovations in agricultural insurance*, The World Bank, (http://www.worldbank.org), Accessed Nov. 2015.

Xi, X., Yan, J., Quan, G., and Cui, L. (2014). "Removal of the pesticide pymetrozine from aqueous solution by biochar produced from brewer's spent grain at different pyrolytic temperatures," *BioResources* 9, 7696-7709.

Zhang, D., and Miller, M. J. (1999). "Polyoxins and nikkomycins: Progress in synthetic and biological studies," *Curr. Pharm. Design* 5(2), 73-99.

Zhang, W., Qiu, L., Gong, A., Cao, Y., and Wang, B. (2013). "Solid-state fermentation of kitchen waste for production of *Bacillus thuringiensis*-based bio-pesticide," *BioResources* 8(1), 1124-1135.