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Pesticides of Botanical Origin: A Promising Tool in Plant Protection

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1. Introduction

Future agricultural and rural development is, to a large extent, influenced by the rapidly increasing food demand of 2.5 billion people expected to swell the world population by 2020. Achieving food sufficiency in a sustainable manner is a major challenge for farmers, agro-industries, researchers and governments (Schillhorn van Veen, 1999). The intensification of agriculture to fulfil food needs has increased the number of insect pest species attacking different crops and as a result the annual production losses of the standing crops. In the past, synthetic pesticides have played a major role in crop protection programmes and have immensely benefited mankind. Nevertheless, their indiscriminate use has resulted in the development of resistance by pests (insects, weeds, etc), resurgence and outbreak of new pests, toxicity to non-target organisms and hazardous effects on the environment endangering the sustainability of ecosystems (Jeyasankar & Jesudasan, 2005). In the recent years the EU has employed a fundamental reform of the Common Agricultural Policy (CAP) highlighting the respect to the environmental, food safety and animal welfare standards, imposing farmlands' cross-compliance with good agricultural and environmental conditions (Schillhorn van Veen, 1999). Due to environmental side effects and health concerns, many synthetic carbamate, organophosphate, and organophthalide pesticides have been banned (Council Directive 91/414/EEC) or are being under evaluation (Regulation 2009/1107/EC OL & Directive 2009/128/EC). On the other hand, industry does not equally sustain the economic cost of research and registration, of all pesticides' chemical classes. The development of nematicides is rarely supported, even though in some cases, such as in the Netherlands, they represent more than 60% of the total pesticides used in agriculture (Chitwood, 2002). This is due to the fact that nematodes are a rather difficult target and the economic cost of research and registration is an enormous hurdle for a prospective new synthetic nematicide to overcome (Chitwood, 2002). As a result, currently there are only few nematicides left in use, and their limited number makes the repeated applications of the same formulation, inevitable. This fact has led to the enhancement nematicides biodegradation in soil (Qui et al., 2004 , Karpouzas et al., 2004, Arbeli & Fuentes, 2007) and the development of resistance in pests. (Meher et al., 2009) These two phenomena are expressed in field as lack of efficacy of the applied pesticides. All the above facts necessitate the urge for new and alternative pest control methods (Chitwood, 2002).

An interesting way of searching for biorational pesticides is screening naturally occurring compounds in plants (Isman, 2006; 2008). Plants, as long-lived stationary organisms, must

resist attackers over their lifetime, so they produce and exude constituents of the secondary metabolism (PSMs), playing an important role in their defence mechanisms. In fact, the phytochemicals' research has its roots in allelochemistry, involving the complex chemical-mediated interactions between a plant and other organisms in its environment (Chitwood, 2002). Among the 500,000 estimated PSMs only 18,000 have been characterised up until 2008. The main groups of PSMs are (i) phenylpropanoids and phenolics, (ii) terpenoids and steroids, (iii) alkaloids and nitrogen compounds. PSMs were used in plant protection from the end of 19th century till the beginning of the Second World War, when synthetic organic pesticides took over. The development of botanicals used as pesticides resulted from two parallel methods: I) the observation of the traditional uses of plants and extracts for cattle and crop protection, followed by checking the efficiency of these practices and identification of the active molecules. The activity of nicotine extracted from tobacco (*Nicotiana tabacum*) and rotenone from Fabaceae *Lonchocarpus nicou* and *Derris elliptica* fall in this category; II) the systematic screening of botanical families followed by biological tests in order to discover the active molecules. Ryanodine, an alkaloid extracted from *Ryania*. sp., and marketed in the United States in 1945, is the result of such prospecting, carried out with a collaboration between Rutgers University and Merck in the early 1940s. Before the Second World War, four main groups of PSMs were used in pest management: nicotine and alkaloids, rotenone and rotenoids, pyrethrum and pyrethrins, and vegetable oils. The commercialization of synthetic pesticides including organochlorides, organophosphates, and carbamates, followed. Research on biopesticides of plant origin was actively pursued again throughout the second half of the 20th century in order to improve their stability or to discover new molecules and new sources of molecules. The development of pyrethrinoids, synthetic molecules analogous to pyrethrum, and neem products (Meliaceae) are characteristic examples of commercial plant protection products based on botanical sources. Botanicals and plant allelochemicals are clearly defined as semiochemicals by Organization for Economic Cooperation and Development (OECD). This definition includes all chemicals involved in species communication (pheromones, but also plant extracts, plant volatiles, and natural oils) and exhibiting pest control activities. The concept of biocontrol agents (BCAs) has recently been preferred to that of biopesticides (Regnault-Roger & Philogène, 2008).

PSMs may have applications in weed and pest management, if developed for use as pesticides themselves, or they can be used as model compounds for the development of chemically synthesized derivatives. Many of them are environmentally friendly, pose less risk to humans and animals, have a selective mode of action, avoid the emergence of resistant races of pest species, and as a result they can be safely used in Integrated Pest Management (IPM) (Isman, 2006). Furthermore, they may be proved suitable and be used as products of choice for organic food production. Extensive is the literature concerning the use of plants' crude or refined extracts in various fields of crop protection (insects, fungi, nematodes, bacteria, weeds). It is mandatory though to attribute the efficacy of botanicals to specific identified constituent compound(s) in order to delineate the mechanisms of bioactivity, biologically and biochemically, and to fully exploit the therapeutic potential of extracts (Akhtar & Mahmood, 1994). This is a short review encompassing the main chemical classes of PSMs that have been used in crop protection focusing on the most recent advances in the chemicals disclosed, their mode of action and their fate in the ecosystem. In addition, we present our research group's findings on biological activities of limonoids and terpenes, representing our step forward to the contribution in this scientific topic. Finally, we examine

the current use of BCAs in pest management and we conclude with the European legislation underlying registration procedures and commercialization potential.

2. Chemical composition

Essential oil components

Essential oils (EOs) are volatile, natural, complex compounds characterized by a string odor and are formed as PSMs by aromatic plants belonging to a number of botanical families, like Myrtaceae, Lauraceae, Lamiaceae, Asteraceae. These chemical volatiles have functions in chemical defence, acting as insecticides, acaricides, avoiding bacterial or fungi phytopathogen colonization, attracting natural enemies of herbivores (Bakali et al., 2008; Yadav, et al., 2008; Karamanoli et al., 2005; Iacobellis et al., 2005; Flamini, 2003; Karamanoli, 2002). Usually they are obtained by hydro-distillation and they comprise terpenes and terpenoids and other aromatic and aliphatic constituents. Terpenes form structurally and functionally different classes of compounds that are formed by coupling different numbers of isoprene units (5-carbon-base; C₅), while terpenoids represent terpenes containing oxygen. The main structural classes of the terpenes are: monoterpenes (C₁₀), sesquiterpenes (C₁₅), hemiterpenes (C₅), diterpenes (C₂₀), triterpenes (C₃₀), tetraterpenes (C₄₀) (Aharoni et al., 2005). The main functional classes of the terpenes (mono-, sesquiterpenes) and aromatic compounds are presented in Table 1. When a molecule is optically active the enantiomers are present in different plants or in some cases they are both present in a racemic form (Bakkali, et al., 2008). EOs are heterogeneous mixtures of single substances, biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Several factors such as phenological age of the plant, percent humidity of the harvested material, and the method of extraction have been identified as possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Lahlou, 2004). Essential oils affect several targets at the same time, because of their great number of constituents; this fact decreases the target organisms' resistance or adaptation. Also, EOs induce cytotoxicity, damage the cellular and organelle membranes, act as prooxidants on proteins and DNA and produce reactive oxygen species (ROS). Such activity is mostly induced by phenols, aldehydes and alcohols. In some cases when photoactive molecules such as furocoumarins, are exposed to activating light, they penetrate the cell without damaging the membranes, proteins and DNA, and then produce radical reactions and oxygen singlet. In some cases essential oils and their components have demonstrated nuclear and cytoplasmic mutagenicity, acting on mitochondria and the respiratory system (Bakkali et al., 2008). The biological activity of EOs and their components on pest insects comprise behaviour and feeding deterrence effects, fumigant toxicity, knockdown activity and lethal toxicity via contact. While these substances are generally active against a broad spectrum of pests, interspecific toxicity of individual oils and compounds is highly idiosyncratic. Perhaps the most attractive aspect of using EOs and their constituents in pest management is their favourable mammalian toxicity and their non-persistence in the environment, for which reason they are exempted from the usual data requirements for registration in the USA (Isman, 2000).

Triterpenoids (Intact and degraded tetranortriterpenoids as well as triterpenoid saponins)

Limonoid triterpenes are known to possess insecticidal and antifungal properties (Akhtar et al., 2008; Carpinella, et al., 2003). Limonoids are metabolically altered triterpenes and have a

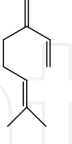
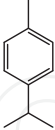
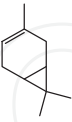
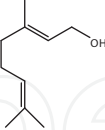
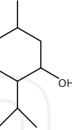
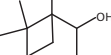
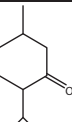

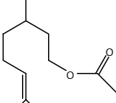
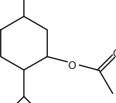
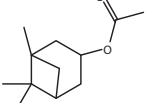
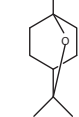

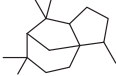
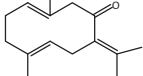
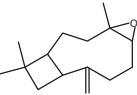


Monoterpenes					
 C ₁₀ H ₁₆ Exact Mass: 136,13 Myrcene	 C ₁₀ H ₁₄ Exact Mass: 134,11 p-Cymene	 C ₁₀ H ₁₆ Exact Mass: 136,13 3,6-Carene	 C ₁₀ H ₁₈ O Exact Mass: 154,14 Geraniol	 C ₁₀ H ₂₀ O Exact Mass: 156,15 Menthol	 C ₁₀ H ₁₈ O Exact Mass: 154,14 Borneol
Hydrocarbon/acyclic	Hydrocarbon/monocyclic	Hydrocarbon/bicyclic	Alcohol/acyclic	Alcohol/monocyclic	Alcohol/bicyclic
 C ₁₀ H ₁₈ O Exact Mass: 154,14 Menthone	 C ₁₀ H ₁₆ O Exact Mass: 152,12 Camphor	 C ₁₂ H ₂₂ O ₂ Exact Mass: 198,16 Citronellyl acetate	 C ₁₂ H ₂₂ O ₂ Exact Mass: 198,16 Menthyl acetate	 C ₁₂ H ₂₂ O ₂ Exact Mass: 196,15 Isobornyl acetate	 C ₁₀ H ₁₈ O Exact Mass: 154,14 Cineole 1,8
Ketone/monocyclic	Ketone/bicyclic	Ester/acyclic	Ester/monocyclic	Ester/bicyclic	Ethers
Sesquiterpenes				Aromatic	
 C ₁₀ H ₈ Exact Mass: 128,06 Azulene	 C ₁₅ H ₂₆ O Exact Mass: 222,20 Centrol	 C ₁₅ H ₂₂ O Exact Mass: 218,17 Germacrone	 C ₁₅ H ₂₄ O Exact Mass: 220,18 Caryophyllene oxide	 C ₁₀ H ₁₀ O Exact Mass: 146,07 Cinnamaldehyde	 C ₁₀ H ₁₂ O Exact Mass: 148,09 Cinnamyl alcohol
Hydrocarbons	Alcohol	Ketones	Epoxide	Aldehyde	Alcohol

Table 1. Chemical classes of essential oils’ components

prototypical structure either containing or deriving from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton. Although hundreds of limonoids have been isolated from various plants, their occurrence in the plant kingdom is confined to plant families of the order Rutales and more abundantly in the families Meliaceae and Rutaceae, and less frequently in Cneoraceae and *Harrisonia* sp. of Simaroubaceae (Manners, 2007). Of the 300 limonoids known today, about one third is obtained from Meliaceae species (*Azadirachta indica* and *Melia azedarach*), also known as meliacins. The structural variations of limonoids found in Rutaceae are less than in Meliaceae and are generally limited to the modification of A and B rings. The limonoids of Meliaceae are more complex with very high degree of oxidation and rearrangement exhibited in the parent limonoid structure (Roy & Saraf, 2006; Connolly & Hill, 2008). Most work has been focused on azadirachtin, a limonoid PSM ($C_{35}H_{44}O_{16}$, a tetranortriterpenoid) of the Indian Neem tree (*Azadirachta indica* L., Meliaceae). The technical grade material of azadirachtin is used for the production of a wide range of commercial formulations exhibiting good efficacy against more than 400 insect species (Akhtar et al., 2008; Lee et al., 1991), mites (Flamini, 2003) and nematodes (Akhtar, 2000; Oka et al., 2007). In India the use of neem (*Azadirachta indica*) extracts in pest management is a part of the traditional practices. Neem is a mixture of more than 100 limonoid compounds, including azadirachtin, salannin, and nimbin and their analogues provoking repellence, feeding deterrence and insect growth inhibition (Schmutterer, 1990). Similar to *A. indica*, *M. azedarach* extracts possesses insecticidal, acaricidal and fungicidal properties and some of the limonoids isolated are 21- β -acetoxymelianol (Ntalli et al., 2010d), meliantriol, melianone, melianol (Lavie & Jain, 1967), meliacin (1-cinnamoyl melianone), meliacarpin (Li et al., 1999) and meliartenin (Carpinella et al., 2002), azedarachin B (Fukuyama et al., 2006) (Table 2). Limonoids do not have direct negative effects on beneficial insects (Charleston et al., 2005; Sengottayan & Sehoon, 2006), a fact that indicates their potential to be combined in biological pest control programmes. Azadirachtin is the mostly ever studied tetranortriterpenoid, which chemical structure required 18 years to solve and its total synthesis took almost 22 years (Morgan, 2009). The mode of action of azadirachtin lays on (i) deterrent effects on chemoreceptors resulting in antifeedancy (ii) effects on ecdysteroid and juvenile hormone titres through a blockage of morphogenetic peptide hormone release (e.g. PTTH; allatotropins) and (iii) direct effects on tissues resulting in an overall loss of fitness of the insect (Mordue & Blackwell, 1993). Within the azadirachtin molecule, the decalin fragment is responsible for the insect growth regulation and development effects observed, while the hydroxyl-furan fragment causes the antifeedant effects more widely observed among target species (Table 2) (Aldhous, 1992). Recently it was proved that azadirachtin provokes a rapid increase in the mitotic index of insect cells, induces the appearance of many aberrant mitotic figures and prevents to some extent the polymerisation in vitro of mammalian tubulin (Salehzadeh et al., 2003). Interestingly the EC_{50} values for various cultured insect cell lines vary from 10^{-10} to 10^{-9} M, by which it is classed as highly toxic, whereas for all mammalian cell lines, values of EC_{50} are 10^{-5} – 10^{-3} M, which places it in the mildly toxic to non-toxic class, and gives a margin of safety in excess of 100-fold between insect and mammalian cells. There is evidence that the difference in toxicity may be due to the ability of mammalian cells to remove azadirachtin from their body (Morgan, 2009). Azadirachtin acute oral LD_{50} in rat is above 5000 mg kg^{-1} and this classifies it by the U.S. Environmental Protection Agency (EPA) in class IV (no mammal toxicity). Additionally it has no effects on skin sensitization, eye irritation, and is not mutagenic (Isman, 1997). Under field conditions azadirachtin and other neem constituents, e.g., salannin, nimbin,

deacetylnimbin, and deacetylsalannin, are not persistent. Three days post field application at five times the dose recommended by the manufacturer, residues of azadirachtin A and B were 0.03 and 0.01 mg/kg, respectively, while residues of salannin (LOQ 0.01 mg/kg) and nimbin (LOQ 0.5 mg/kg) were not detectable. Sunlight photodegradation is the main factor influencing the rate of its disappearance after greenhouse treatment while tomato epicuticular waxes double the photodegradation rate of a commercial formulation (Caboni et al., 2006; 2009). Besides limonoids, also the quassinoids and saponins fall in the PSMs' category of triterpenoids, being though much less studied (Table 2). Quassinoids, the bitter principles of the Simaroubaceae family (*Quassia amara*, *Cassia camara* and *Picrasma excelca*), are a group of structurally complex and highly oxygenated degraded triterpenes (Sarais et al., 2010). They are divided into five groups according to their basic skeleton, C-18, C-19, C-20, C-22 and C-25. In recent years, attention has been focused on quassinoids because several of them have shown promising biological activities phytotoxic, antifeedant, insecticidal (Almeida et al., 2007). Quassinoids act against insects, nematodes and weeds (Koul, 2008; Powell et al., 1998; Leskinen et al., 1984; Chitwod, 2002; Lin et al., 1995). In nematodes quassinoids acts as noncompetitive antagonists of the ionotropic GABARs to stabilize the closed conformation of the channel, resulting in the inhibition of the action of GABA (Kuriyama et al., 2005). No data are currently available concerning quassinoids fate in the environment. The plant-derived saponins are triterpene glycosides obtained from the soap bark (or soapbark) tree, *Quillaja saponaria* (Quillajaceae) as well as various other plant species of the families Alliaceae, Asteraceae, Polygalaceae and Agavaceae. Their side chains

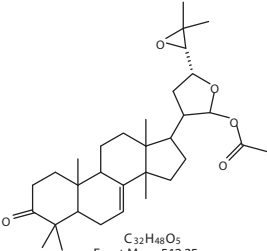
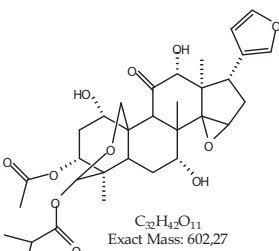
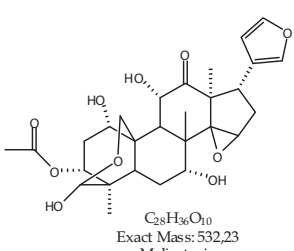
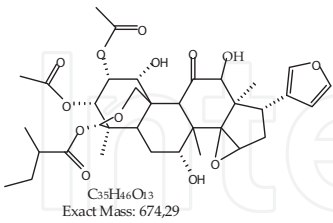
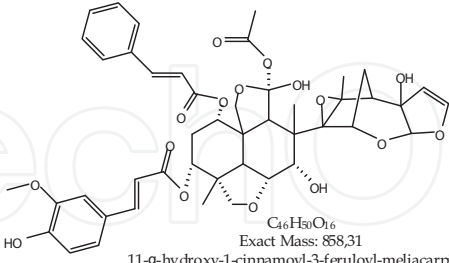
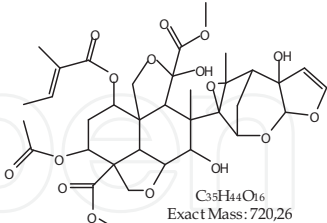
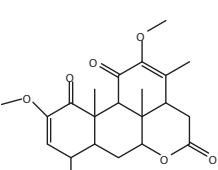
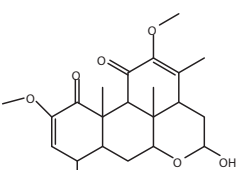
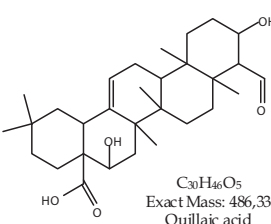
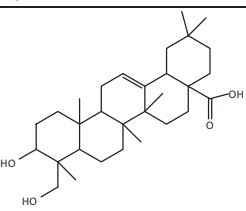
Triterpenoids			
 C ₃₂ H ₄₈ O ₅ Exact Mass: 512,35 21-β-acetoxy-melianone	 C ₃₂ H ₄₂ O ₁₁ Exact Mass: 602,27 Azedarachin B	 C ₂₈ H ₃₆ O ₁₀ Exact Mass: 532,23 Meliartenin	
 C ₃₅ H ₄₆ O ₁₃ Exact Mass: 674,29 Trichilin A	 C ₄₆ H ₅₀ O ₁₆ Exact Mass: 858,31 11-α-hydroxy-1-cinnamoyl-3-feruloyl-meliacarpinin	 C ₃₅ H ₄₄ O ₁₆ Exact Mass: 720,26 Azadirachtin	
Quassinoids		Saponins	
 C ₂₂ H ₂₈ O ₈ Exact Mass: 388,19 Quassin	 C ₂₂ H ₃₀ O ₆ Exact Mass: 390,20 Neoquassin	 C ₃₀ H ₄₆ O ₅ Exact Mass: 486,33 Quillaic acid	 C ₃₀ H ₄₈ O ₄ Exact Mass: 472,36 Hederagenin

Table 2. Structures of intact and degraded triterpenoids as well as triterpenoid saponins.

of hydrophilic carbohydrates provide them with surfactant properties, but they possess also significant antifeedant, fungicidal and nematicidal properties (Chitwood, 2002; Koul, 2008; Duke et al., 2003; D'Addabbo et al., 2006; 2010; Ribera et al., 2008; Martin & Magunacelaya, 2005). Saponins disrupt also membranes (Majak, 1992).

Glucosinolates and Isothiocyanates (Brassicaceae)

Glucosinolates (GLSs) are sulphur and nitrogen containing PSMs produced by “mustards” (*Brassica* & *Sinapis* sp.) as well as other genus of the Capparales order. Glucosinolates are an important and unique class of secondary plant products containing b-D-thioglucose and sulphonated oxime moieties. These include thioglucosides, characterized by side chain with varying aliphatic, aromatic and heteroaromatic carbon skeletons. Glucosinolates get inverted into various degradation products (isothiocyanates, thiocyanates, indoles etc.), when vegetables containing them are cut or chewed, because during this process they come in contact with the enzyme myrosinase that hydrolyses them. By incorporating glucosinolate-containing plant material in soil their bioactive hydrolysis products, named isothiocyanates (ITCs) are released. These products can be used to control soil pests and weeds - a practice known as biofumigation (**Figure 1**). This practise is considered an ecological substitution of the soil fumigation with toxic fumigants such as MeBr, used in the past to suppress soil fungus, bacteria, nematodes and weeds, since it is considered fully biodegradable and less toxic (Vig et al., 2009). ITCs trigger the plant's defence mechanism, produce toxins that kill the target organisms, and produce defensive barriers around the roots of the host plant thus preventing the harmful fungi to enter the host. In fungus ITCs inhibit the oxygen uptake through the uncoupler action of oxidative phosphorylation in mitochondria, they inhibit the coupling between the electron transport and phosphorylation reactions and eventually hinder the ATP synthesis. In bacteria ITCs inactivate various intracellular enzymes by oxidative breakdown of -S-S- bridges and they obstruct ATP synthesis in cells through uncoupler action of oxidative phosphorylation in mitochondria. In insects ITCs inactivate the thiol group of essential enzymes, alkylate the nucleophilic groups of biopolymers like DNA and act as uncouplers accelerating the respiration, which needs more ATP as source of energy, while at the same time ATP production is blocked. This causes exhaustion of stored energy sources which finally leads to death of the pest. In weeds they inhibit seed germination by interfering with protein synthesis and formation of phosphorylated sugars, or inhibit plant enzyme activity (Vig et al., 2009). The ITCs' sorption, degradation or loss from soil mechanisms is fundamental for developing effective, but environmentally benign biofumigation strategies. Effective biofumigation relies on maximum hydrolysis of the glucosinolates in the plant tissue to generate high isothiocyanate concentrations in the soil after incorporation. This is favoured by maximum cell disruption, by addition of water, and a high soil temperature. Residual glucosinolates are very weakly sorbed, readily leached and are microbially degraded and mineralised in soil. In contrast, isothiocyanates are strongly sorbed by the organic matter in soil, react strongly with nucleophilic groups present in soil, and are prone to volatilization losses and microbial degradation and mineralisation minimizing the risks of persistence in the environment or leaching (Gimsing & Kirkegaard, 2009). During the recent years extensive reviews have concerned the chemical ecology of various Brassica towards parasitoids, predators, herbivores and nematodes emphasizing on GLSs and ITCs, their potential of integration in insect-pest management, the physiological and biochemical implications

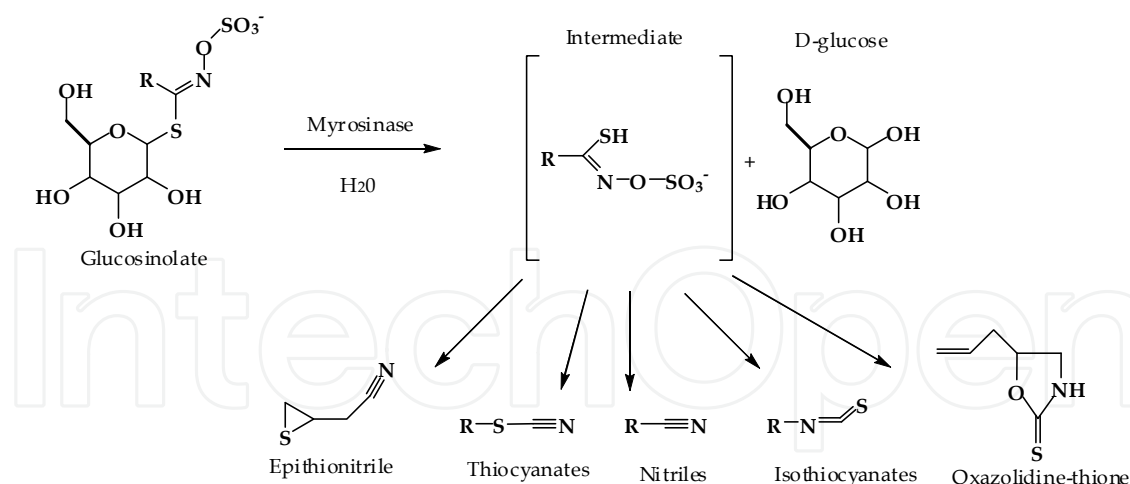


Fig. 1. A hydrolysis scheme of sinigrin under the action of myrosinase and respective degradation productions

underlying hydrolysis mechanisms (Ahuja, et al., 2010; Monfort et al., 2007; Kissen et al., 2009; Agerbirk et al., 2009).

Cyanogenic glycosides

Cyanogenic glucosides constitute a limited number of amino acid derived PSMs known to be present in more than 2500 plant species. This group of compounds is considered to play an important role in plant defence against herbivores due to their bitter taste and release of toxic hydrogen cyanide. Upon tissue disruption (e.g. chewing insects) the cyanogenic glucosides are released from the vacuoles and hydrolyzed by specific β -glucosidases to yield glucose, a ketone or an aldehyde and toxic HCN. This process is known as cyanogenesis and serves to facilitate a rapid HCN release (Figure 2) that suppress insects, fungus, nematodes and weeds (Zagrobelny et al., 2004; Morant et al., 2007; Bjarnholt et al., 2008; Carlsen & Fomsgaard, 2008). Cyanogenic glycosides, through the action of cyanide, prevent oxygen utilization by the inhibition of cytochrome oxidase (Majak, 1992).

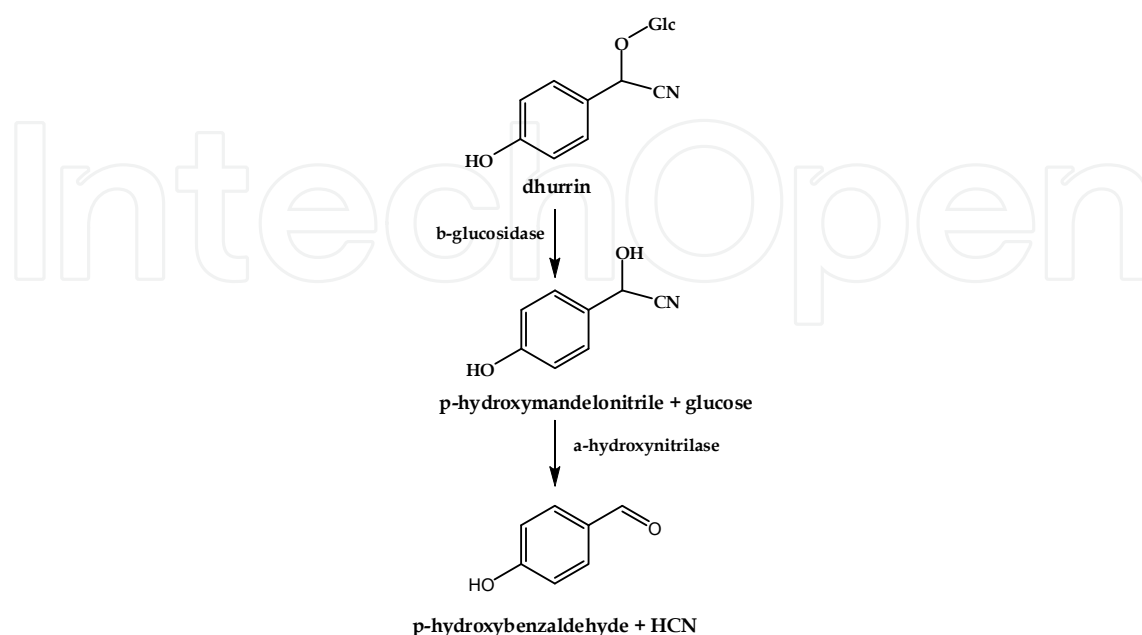


Fig. 2. Cyanogenesis (adapted by Morant et al, 2007)

Alkaloids

Alkaloids are PSMs containing nitrogen atoms, and derive from various botanical families amongst which the Solacaneae. Nicotine is undoubtedly the oldest alkaloid used in agriculture as well as the one of the first molecules used as insecticide (Table 3). It is an acetylcholine mimic binding to postsynaptic receptors and interfering with the transmission of signals in nerves, leading to a continuous firing of the neuroreceptor. This overstimulation leads to depression the central nervous system. It acts predominately through the vapour phase and to a less degree through stomach and contact. Nicotine's high toxicity to humans limited its use as a pesticide (Regnault-Roger & Philogène, 2008). Bio-transformations of nicotine, involving activation reactions and detoxification mechanisms, have led to neonicotinoids, representing the current major class of insecticides of outstanding potency, systemic action and low toxicity to mammals (Tomizawa & Casida, 2008). Other alkaloids falling in the same category are veratrine and cevatrine, the major components of sabadilla (*Schoenocaulon officinale* Grey) seeds, which are mainly used to control thrips, but recently resistance issues have broken up (Humeres & Morse, 2006). Sabadilla alkaloids possess, like pyrethrins, a neurotoxic activity by slowing the shutting of Na⁺ channels and disturbing membrane depolarization. They cause paralysis before death. They are contact and nonsystemic insecticides, readily degraded in air and sunlight and are not considered hazardous to non target organisms (Copping, 2004). Ryanodine and its derivative, the dehydro-ryanodine, are extracted from *Ryania speciosa* (Liliaceae) naturalizing the Amazonian basin. Ryanodine acts against insects by interfering with the nerve impulse at the Ca²⁺ channel level and provoking a sustained contraction of the muscles and paralysis. This mode of action has inspired synthetic chemistry, and ryanodine receptors currently represent molecular targets for novel pest control chemicals (Sattelle et al., 2008). The toxicity of *Ryania* extracts towards mammals and fish has precluded its continuing use. Finally 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) is a sugar analogue, pyrrolidine alkaloid contained in the genera *Lonocarpus* and *Derris*, exhibiting nematocidal activity. It is downwardly mobile in plant phloem, applications on plant foliar decrease galling in roots, but its mode of action is under investigation (Chitwood, 2003).

Phenolics – Flavonoids

Phenolics are toxic to insects, fungi, bacteria, nematodes and weeds (Koul, 2008; Carlsen & Fomsgaard, 2008; Simmonds & Stevenson, 2001; Popa et al., 2008; Wu et al., 2001; Simmonds, 2003; Chitwood, 2002). Flavonoids, a major class of phenolic compounds, are distributed widely in vascular plants and Bryophytes, and ca. 5,000 kinds have been reported to possess feeding attractant and deterrent properties (Iwashina, 2003). Rotenone (Table 4), a flavonoid, present in plants of the genus *Derris* or *Lonchocarpus* (Leguminosae) is, with the alkaloid nicotine, one of the oldest insecticides used all over the world. The principal commercial product of the botanical insecticide rotenone comes from Cube resin, a root extract of *Lonchocarpus utilis* and *Lonchocarpus urucu*. Although rotenone is the primary major constituent in insecticides containing these preparations, a second isoflavone, deguelin, also possesses similar biological properties (Table 4) (Dayan et al., 2009; Caboni et al., 2004). Rotenone inhibits cellular respiration and energetic metabolism at the level of the mitochondrial respiratory chain. It is easily biodegradable and its half life under field conditions is 5 to 7 h (Cavoski et al., 2007). Initially it has been characterised moderately toxic to mammals, but it eventually links to Parkinson's disease (Giasson & Lee, 2000) and recently rotenone has not been included in Annex I of Council Directive 91/414 EEC

(2008/317/EC) and authorizations are withdrawn according to EU legislation. Finally, karanjin (3-methoxy-2-phenylfuro[2,3-h]chromen-4-one) is a furanoflavonol obtained from *Derris* that acts as acaricide and insecticide (Pavela, 2009).

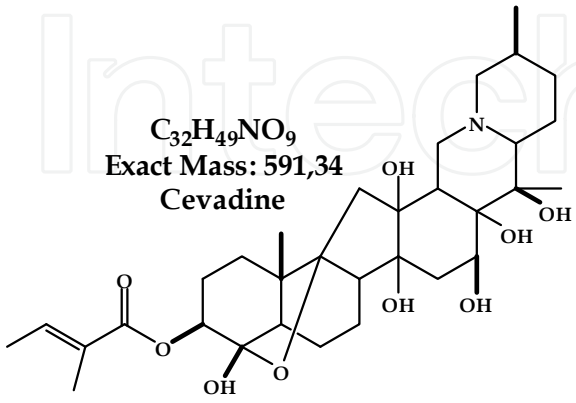
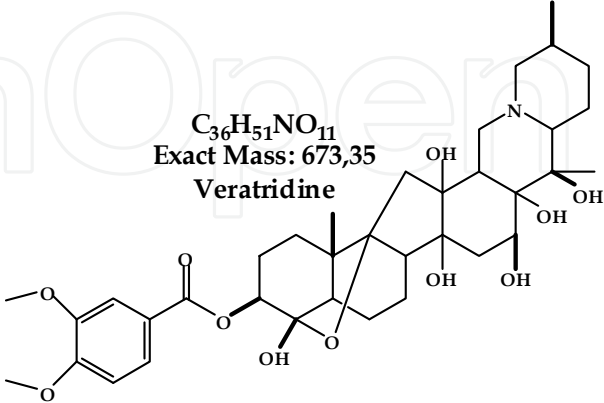
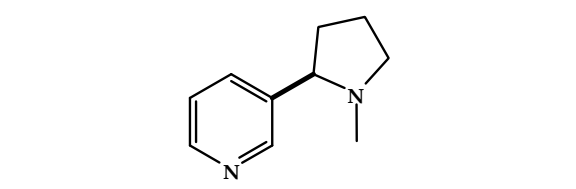
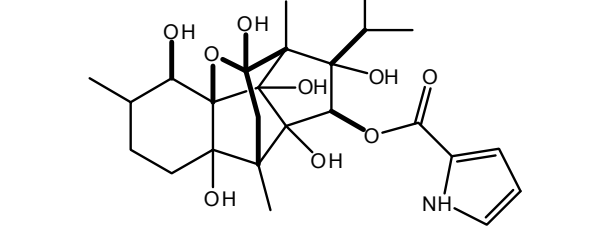
Alkaloids	
<div><p>C₃₂H₄₉NO₉ Exact Mass: 591,34 Cevadine</p></div>	<div><p>C₃₆H₅₁NO₁₁ Exact Mass: 673,35 Veratridine</p></div>
<div><p>C₁₀H₁₄N₂ Exact Mass: 162,12 Nicotine</p></div>	<div><p>C₂₅H₃₅NO₉ Exact Mass: 493,23 Ryanodine</p></div>

Table 3. Chemical structures of nicotine and other alkaloids extracted from *Ryania* and *Sabadilla* species

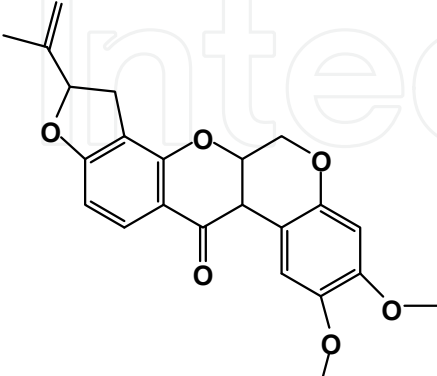
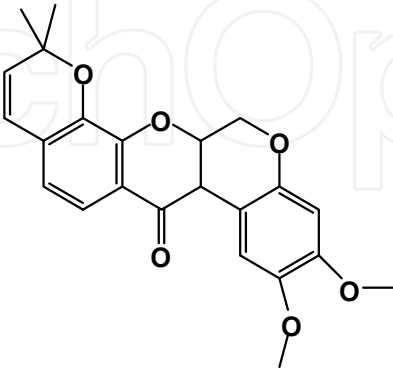
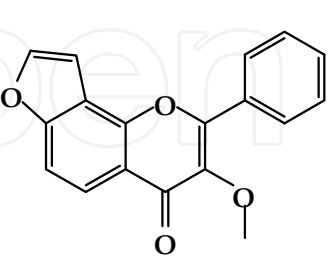
Flavonoids		
<div><p>C₂₃H₂₂O₆ Exact Mass: 394,14 Rotenone</p></div>	<div><p>C₂₃H₂₂O₆ Exact Mass: 394,14 Dequelin</p></div>	<div><p>C₁₈H₁₂O₄ Exact Mass: 292,07 Karanjin</p></div>

Table 4. The major flavonoids contained in *Derris* or *Lonchocarpus*

Polyacetylenes & Polythienyls

They are substances present in *Tagetes* species, commonly “marigolds”, of the botanical family Asteraceae. Polyacetylenes and Polythienyls possess insecticidal and nematicidal properties (Chitwood, 2002; Wat et al., 1981)

Pyrethrum

Pyrethrum is a powder obtained by crushing dried flowers of daisies belonging to the family of Asteraceae such as *Chrysanthemum*. spp., *Pyrethrum*. spp., and *Tanacetum*. spp. Pyrethrum is a mixture of six esters, pyrethrins I (**Figure 3**) and II, (the most abundant), cinerin I and II, and jasmoline I and II. Pyrethrins control a wide range of insects and mites binding to Na⁺ channels and prolonging their opening. The insect presents hyperactivity followed by convulsions and finally it dies. The rapid action of pyrethrins is called knockdown effect. Pyrethrins have a relatively low toxicity toward mammals but toxicity is mentioned for nontargeted species, especially fish and bees. However, their great instability to light, air, and moisture reduce considerably the risks related to its use. Currently, pyrethrum is limited and costs have risen in recent years, making it inaccessible to less affluent societies (Isman, 2008).

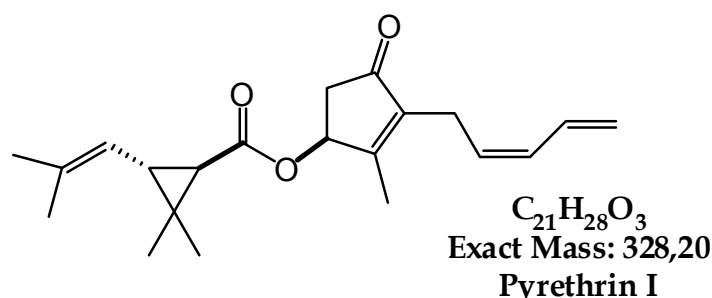


Fig. 3. Chemical structure of Pyrethrin I

Organic Acids

Vegetable oils contain large and heterogeneous quantities of fatty acids, saturated or unsaturated, with medium to long esterified carbon chains, and esters of fatty acids with high molecular weight. They develop toxicity by inhalation and contact suffocating the insect by forming an impermeable film upon the cuticle. Some compounds penetrate through the cuticle, disrupt cellular membrane, and uncouple oxidative phosphorylation. Some fatty acids, such as oleic (C18), have their own insecticidal activities, whereas undecylenic (C11) acid has a lower toxicity, but increases the activity of other insecticidal compounds by potentiation (Regnault-Roger & Philogene, 2008).

Others

The PSMs produced by many species in the genus *Piper* are called piperamides and they are characterised by insecticidal activity. Piperamides provoke contact toxicity, repellent and antifeedant activities and in a biochemical level act as neurotoxins. They quickly degrade under full sunlight (Scott et al., 2008). Capsaicin is obtained from the genus *Capsicum* such as chili peppers (*Capsicum frutescens*, Mill.) and is characterised by nematicidal, insecticidal and insect repellent properties (Neves et al., 2009; Dayan et al., 2009; Edelson et al., 2002). As an emerging biocide very little data is available on the environmental fate of capsaicin but initial assessment suggests it will bind to sediments (Tomas & Brooks, 2010).

3. Greek plants as a source of botanical pesticides: *Results of our ongoing research*

Botanicals have been in use for a long time for pest control offering an alternative approach to crop protection. Mediterranean area reserves abundance in plants exhibiting bioactive properties. Improvement in our understanding of plant allelochemical mechanisms of activity offer new prospects for using these substances in crop protection. In the frame of our research interests aiming at the plant chemical defense and the investigation for alternative chemical agents to control plant diseases and pests, we evaluated the activity of various indigenous plants, grown in the Mediterranean region against bacteria, insect, fungi and nematodes. The chemical composition of plants was investigated and determined in order to further be correlated with the biological activity of the tested plants.

Bacteria, fungi & terpenes

Studies on the activity of plant EOs and their terpenoid constituents against phytopathogenic or foodborne bacteria and fungi are receiving increased interest in scientific annals, as alternatives to synthetic pesticides and as key components of IPM. In our first attempts for eco-friendly methods to control phytopathogenic bacteria we investigated the role of PSMs obtained from plants indigenous or introduced, wild or cultivated in Greece. As a first trigger, we found that bacterial population on plant phyllosphere differ significantly from species to species and also between the same species. We also found a correlation between bacterial colonization of plant phyllosphere and presence of secondary metabolites (Karamanoli et al., 2000; 2005). Results of this work provided evidence of the allelopathic effect of PSMs in the field against epiphytic bacteria and specifically Labiatae aromatic plants are protected against them by both EOs and leaf surface phenolics. Interestingly, the highest colonized lavender had the lowest EO content, compared to other aromatics (oregano, rosemary and sage) and this oil exhibited weak antibacterial activity. Particularly for EOs, another important factor is the amount of isoprenoid compounds that they contain, with phenols and oxygenated terpenoids generally, being the most effective (Karamanoli et al., 2000). Building on our team's previous work, the antibacterial activity of secondary metabolites (EOs, surface phenolics and leaf tissue extracts) from 19 Mediterranean species were evaluated and correlated with their chemical class and composition. Proper analytical techniques (hydrodistillation and LL extraction) were used for their isolation, followed by GC-MS analysis for chemical composition identification and bioassays or greenhouse experiments for antibacterial activity (MIC and MBC) estimation (Karamanoli et al., 2005). All aromatic plants were found active, with PSMs of *Oreganum* spp and *Thymus* spp being the most toxic. Furthermore, the activity of their main constituents was evaluated. Results showed carvacrol as the most potent compound ($\text{MBC}=0.4\text{mg mL}^{-1}$) against all tested eleven bacteria strains. Generally cyclic alcohols and ketones exhibited high activity, followed by long chain alcohols and hydrocarbons with less or no activity. Oregano oil, rich in carvacrol and thymol, also proved effective as an antibacterial biocontrol agent in field experiments. Consequently, the antibacterial activity of aromatic plants may have applications in agriculture for developing efficient biocontrol agents from these plants, with the prerequisite to overcome their limited persistence and formulation requirements. .

Similarly, we found a correlation between chemical composition of EO, isolated from *Pistachia* spp, and septorioses caused by the pathogenic fungi *Septoria pistaciarum*. Terpinen-

4-ol was identified as the most active constituent of the e.o. against *S. pistaciarum* and determined at high concentration only in the EO of the tolerant plants (Douka et al., 2005).

Insects & terpenes

Insecticidal effects of plant EOs and individual terpenes against disease vectors and insect pests consists a well studied case (Isman, 2000). Specifically, contact and fumigant activity of plant EOs against stored product pests have been reported, but the relationship between their chemical composition and their activity is always needed to be determined, in order the results to find further practical applications. Studies were performed aiming to evaluate the insecticidal activity (LC_{50}) of EOs obtained from the aromatic plants lavender (*Lavandula hybrida* Rev, Lamiaceae), rosemary (*Rosmarinus officinalis* L, Lamiaceae) and eucalyptus (*Eucalyptus globulus* Labill, Myrtaceae) and their main constituents against *Acanthoscelides obtectus*, Say (Papachristos et al., 2004). Strong insecticidal activity was found, and oxygenated monoterpenes were predominated over hydrocarbons. All EOs tested exhibited strong activity against *A. obtectus* adults and variability in their activity and chemical composition due to different plant part, season and insect sex was substantiated. Among 16 of the principal components of the EOs tested, the most active were terpinen-4-ol, camphor, 1,8-cineol and verbenone, followed by linalool ($LC_{50}=0.8-7.1\text{mg L}^{-1}\text{ air}$), while linalyl and terpinyl acetate were active only against adult males. Overall, intact EOs are more potent in controlling *A. obtectus* than are their main constituents, the monoterpenoids.

Nematodes & terpenes

Phytonematodes are among the most notoriously difficult crop pests and their control is achieved mainly with cultural practices, crop rotation, and resistant cultivars, combined with a few available chemical nematicides that are still authorized. The need for discovering less toxic and environmentally acceptable substitutes for commercial nematicides is amplified, creating a significant market opportunity for alternative products such as biorationals. Essential oils and their components such as thymol, carvacrol, pulegone, limonene, anethole, geranial, and Artemisia ketone have been identified with nematocidal activity (Oka, 2000). As part of our ongoing effort towards the study of natural substances with biologically interesting properties, we have also focused on various plant species of Greek flora as sources of nematocidal compounds. We tested the paralysis activity of the EOs obtained with hydrodistillation from 15 botanical species on root knot nematodes (RKN), as well as the individual and paired activity of 23 pure terpenes, identified as components of the tested EOs (Ntalli et al., 2010b; 2010c). The activity of EOs against *M. incognita* was found to decrease in the order *O. vulgare*, *O. dictamnus*, *M. pulegium*, *M. officinalis*, *F. vulgare*, *P. anisum*, *E. meliodora*, and *P. terebinthus*. Among terpenes, the oxygenated compounds (alcohols and ketones) exhibited in general higher activity than hydrocarbons. The activity of the nematocidal terpenes was found to decrease in the order L-carvone, pulegone, *trans*-anethole, geraniol, eugenol, carvacrol, thymol, terpinen-4-ol, estragole and γ -eudesmol. Furthermore, the aromatic aldehyde benzaldehyde, found as a component of the EO from *E. meliodora*, exhibited the highest activity ($EC_{50}=9\text{ }\mu\text{g mL}^{-1}$). Our results, as well as results from similar studies (Lahlou, 2004; Jiang, 2009), cohere to the fact that the contribution of each ingredient compound to the overall activity of an EO is a complicated pattern of interactions. It is possible that they may act together synergistically or antagonistically to contribute to the toxicity of the totality of the tested oil. Notably, it was confirmed that

“inactive” constituents may have some synergic effect on the “active” constituents and that, although not active individually, their presence is necessary to achieve full toxicity. Generally according to our findings, the EC_{50} values of the individual terpenes, measured at the same concentrations as expected in the EO at their EC_{50} value, was not as high as the activity of the corresponding EO indicating apparently, evidence of components interactions within the oil. To corroborate the role of individual constituents toward the synergistic and antagonistic actions, among each other, artificial blends were further tested for their activity. The terpenes’ pairs exhibiting high binary action (synergism), in decreasing order were: *trans*-anethole/geraniol, *trans*-anethole/eugenol, carvacrol/eugenol and geraniol/carcacrol (Ntalli et al., 2010c). Understanding the interactions among the individual constituents of a natural extract/mixture provide significant information for further development of new plant protection products of environmental friendly approach. Active synergistic mixtures exhibiting high nematocidal activity could be used in artificial blends constituting promising agents of pest management. For this, further studies, concerning the interactions dependence on terpenes concentration and their ratios in mixtures, as well as the underling responsible biological mechanisms of terpenes provoking J2 paralysis, are in process. Additionally, further investigation is required in order to evaluate interaction effects between nematocidal and non nematocidal terpenes.

Nematodes & limonoids

The plant family *Meliaceae* (mahogany family) has received much attention especially because of the presence of limonoid triterpenes many of which are known for their insecticidal properties (Akhtar et al., 2008). The majority of research has been focused on azadirachtin, a limonoid secondary metabolite ($C_{35}H_{44}O_{16}$, a tetranortriterpenoid) of the Indian Neem tree (*Azadirachta indica* L., *Meliaceae*). The technical grade material is used for the production of a wide range of commercial formulations exhibiting good efficacy against more than 400 insect species. Although several reports suggest neem products, such as seed powder, seed kernel powder, seed cake powder, dry leaf powder and aqueous neem extracts, to exhibit good efficacy against root-knot nematodes the results were rather contradictory. We proved that azadirachtin (Neemazal® 1%EC, Intrachem Hellas) acts against RKN at very high concentrations both concerning paralysis effects and biological cycle arrest (12.8 mg a.i. L^{-1} and 30.72 μg a.i. g^{-1}) and that the recommended dose for nematodes’ control in field does not provide adequate control (Ntalli et al., 2009). In the frame of our research on limonoids against RKN we then explored the nematocidal activity of the botanical species *Melia azedarach* L. (*Meliaceae*), which is naturalized in Greece. Chinaberry demonstrated biofumigant properties when incorporated as pulverized fruits in *M. incognita* infested soil to be tested for its effect on nematode life cycle ($EC_{50}=0.34$ % w/w). First we distinguished for paralysis activity amongst the polar and non-polar fractions of the fruits extracts, and we established proper extraction procedures for maximum yields’ obtainment. The activity of *M. azedarach* against RKN was attributed to the defatted methanol extract (polar fragment) of the fruit and this was used in extends in pot experiments to study its effect on the RKN biological cycle arrest. The EC_{50} values were calculated for all experiments. Four days (96 h) of juveniles immersion in a 0.03 % w/v *Melia* Methanol Extract (MME) solution paralysed half the population tested ($EC_{50}=0.03$ % w/v), while the EC_{50} value calculated for the pot experiments was 0.916 % w/w (Ntalli et al., 2010a). Further studies on chemical characterization and biological activity of the defatted

methanol extract *M. azedarach* L revealed that the nematicidal activity does not lay in its limonoids' contents (3- α -tigloylmelianol, melianone, 21- β -acetoxy-melianone, methyl kulonate) but in its organic acids, aldehydes and alcohols (Ntalli et al., 2010e). The aldehyde furfurale was the foremost nematicidal principle, exhibiting activity similar to that of the commercial nematicide fosthiazate, both after J2 immersion in test solutions and after exposure to its vapours (fumigant activity). Furfurale is already known to possess high nematicidal fumigant activity against *M. incognita*, tested in greenhouse and microplot conditions (Rodrigues-Kabana et al., 1993) but no correlation had been ever made so far with Chinaberry. The nematicidal activity of *M. azedarach* as well as its contents in furfurale, reported by our group for the first time, reveals this species' nematicidal-biofumigant toxicity and its potency of incorporation into IPM programs. Because phytonematodes live in soil or within plant roots, the target of any chemical nematicide often resides at a fair distance away from the site of application. The fumigant activity of a nematicide is a rather important property enhancing its activity in the non treated soil layers.

These results combined with literature cited reports on activity of natural products of plant origin against various pests show their suitability to be considered as potential biopesticides. Our current studies are focused on the mode of action of these natural products specifically within nematodes' body, while further studies on the formulation and on the environmental fate are needed.

4. Current trend and future prospective

In this review we are aiming to explore the ability of the nature and the abundant resources for chemicals available for plant defence and suitable in pest management for crop protection. BCAs have long been touted as attractive alternatives to synthetic chemical pesticides for pest management because botanicals reputedly pose little threat to the environment and to human health. The body of scientific literature documenting bioactivity of plant derivatives to pests continues to expand rapidly, yet only a handful of botanicals are currently used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products. Several factors appear to limit the success of botanicals, most notably regulatory barriers and the availability of competing products (newer synthetics, fermentation products, microbes) that are cost-effective and relatively safe compared with their predecessors (Isman, 2006). Botanical pesticides presently play only a minor role in crop protection; increasingly stringent regulatory requirements in many jurisdictions have prevented all but a handful of botanical products from reaching the marketplace in North America and Europe in the past 20 years (Isman, 2008).

Thus the regulation of natural products as crop-protection agents may have to undergo the same procedure as for a conventional chemical product. Each country or region has a different approach; for example, the USA EPA separates pesticides into two general categories: conventional chemical pesticides and biochemical/microbial pesticides, while Europe follows the OECD definition of biopesticides (rather than natural products), which includes pheromones, insect and plant growth regulators, plant extracts, transgenic plants and macro-organisms. The requirements, risk assessments and encouragement for such products differ from region to region (Neale, 2000). Concerted efforts are hoped to remove limitations in biopesticides raw material availability, potency variations, standardization of extraction methods, quality control, shelf life and improved bioefficacy (Mendki et al., 2001).

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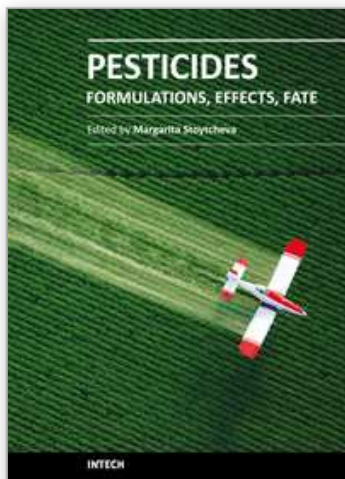
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This book provides an overview on a large variety of pesticide-related topics, organized in three sections. The first part is dedicated to the "safer" pesticides derived from natural materials, the design and the optimization of pesticides formulations, and the techniques for pesticides application. The second part is intended to demonstrate the agricultural products, environmental and biota pesticides contamination and the impacts of the pesticides presence on the ecosystems. The third part presents current investigations of the naturally occurring pesticides degradation phenomena, the environmental effects of the break down products, and different approaches to pesticides residues treatment. Written by leading experts in their respective areas, the book is highly recommended to the professionals, interested in pesticides issues.

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