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# Acaricides – Biological Profiles, Effects and Uses in Modern Crop Protection

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## 1. Introduction

Acaricide is a pesticide designed to control harmful species of mites (Acari)<sup>1</sup>. In crop protection practices, acaricides are used against phytophagous mites, pests causing economic injuries to agricultural crops and ornamental plants. Until mid-twentieth century, in agroecosystems of low-level productivity, phytophagous mite populations usually stayed below economic injury levels, due to natural regulation by predatory mites and insects, their natural enemies. The concept of secondary pest outbreak was introduced on spider mites (Tetranychidae), the most important plant-feeding mites, as a paradigm. Advances in agricultural production after World War II, based on the extensive use of pesticides and fertilizers, irrigation and other cultural practices, induced increase in spider mite populations far above economic threshold (Huffaker et al., 1970; McMurtry et al., 1970; Jeppson et al., 1975; Metcalf, 1980). Grown under favourable conditions, host plants became high quality food sources for the mite pests, which gave rise to outbreaks of their populations and made it possible to compensate for the losses caused by predators' activity. Moreover, widespread use of neuroactive insecticides (synthetic organic compounds used against insects as target pests, but toxic to other non-target insect and mite species as well) destroyed spider mite predators, generally more susceptible than their prey; on the other hand, heavy selection pressure by neuroactive insecticides caused emergence of tetranychid mite populations resistant to these compounds. Besides the resistance of spider mites and the elimination of their predators, as the primary causes, outbreaks are influenced by sublethal effects of pesticides on behaviour and physiology of pests and/or predators (Metcalf, 1980; Hardin et al., 1995; Dutcher, 2007).

Spider mites, mostly polyphagous species, are common pests in modern agroecosystems worldwide, and some of them are among the most important crop pests. After Tetranychidae,

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<sup>1</sup> Mites (subclassis Acari), morphologically and ecologically very diverse assemblage of tiny invertebrates, belongs to class Arachnida (together with spiders and scorpions), subphylum Chelicerata and phylum Arthropoda. The arthropods also include insects, from which mites differ, beside being eight-legged animals (insects are hexapods) by the lack of true head and conspicuous body segmentation. There are some 50.000 mite species known today, but it is estimated that the true number is 20 times higher. Besides agricultural pests and their natural enemies (predators), mites include species of medical and veterinary importance (house dust mites, scabies mites, ticks), while the species living in soil and water are important environmental indicators.

the second most important mite pests are gall and rust mites (Eriophyoidea), while the other economically harmful species can be found among false spider mites (Tenuipalpidae), tarsonemid mites (Tarsonemidae) and acarid mites (Acaridae). Phytophagous mites feed on the liquid content of plant cells, thus disrupting the physiology of a host plant and causing various damages to plant tissues and organs, while some of the species can also act as vectors of plant viruses. In spite of relatively small size (100-400  $\mu\text{m}$ ), plant-feeding mites can cause considerable crop yield and quality losses, because they have short life span and under favourable conditions their populations quickly reach high abundance (Helle & Sabelis, 1985a,b; Lindquist et al., 1996; Zhang, 2003; van Leeuwen et al., 2010).

The use of acaricides has increased substantially over the past half of the 20th century. Since the first serious and widespread outbreaks of spider mites populations, during the 1950s, organophosphorous and other neuroactive insecticides were replaced by specific acaricides i.e. compounds exclusively or primarily effective against mites. Several generations of structurally diverse synthetic acaricides, directed against various biochemical and physiological targets, have been commercialized until now. Besides specific acaricides, a number of insecticides with considerable acaricidal activity (pyrethroids, avermectins, benzoylureas) have also been used, while some older neuroactive compounds are still available for the control of phytophagous mites (Jeppson et al., 1975; Knowles, 1997; Dekeyser, 2005; van Leeuwen et al., 2010). Most of the modern acaricides exert their effects through disruption of respiratory processes. Another approach in the development of synthetic acaricides launched compounds that act on growth and development (Dekeyser, 2005; Krämer & Schirmer, 2007). On the other hand, various natural bioactive products with acaricidal activity (botanical and microbial pesticides, essential oils, horticultural spray oils, mycopesticides) have become important alternatives to synthetic acaricides (Beattie et al., 2002; Copping & Duke, 2007; Faria & Wraight, 2007).

Acaricide resistance in phytophagous mites is a seriously increasing phenomenon, especially in spider mites which have a remarkable intrinsic potential for rapid evolution of resistance (Croft & van de Baan, 1988; van Leeuwen et al., 2009). Their populations have often developed a very high degree of resistance to a newly introduced compound after few years of use, with cross-resistance to other compounds with the same mode of action. According to APRD (*Arthropod Pesticide Resistance Database*) more than 700 cases of acaricide resistance in phytophagous mites have been reported. About 93% of these reports refer to spider mites resistance, and almost a half of spider mite resistance cases is related to the twospotted spider mite (*Tetranychus urticae*), highly polyphagous species, one of the most important pests in greenhouses throughout the world (Whalon et al., 2008, 2010). Therefore, there is a continual need for development and application of new acaricides with novel biochemical modes of action, but also for optimization of their use in order to prevent or delay the evolution of resistance and prolong their life span (Dekeyser, 2005). Considering biorational pest control as key approach to modern crop protection (Horowitz et al., 2009) new acaricides should be selective, that is, effective against the target pests and compatible with their natural enemies. Moreover, these compounds must be safe products with respect to human health, beneficial and non-target organisms (mammals, birds, earthworms, bees, aquatic organisms) and the environment in order to meet the regulatory requirements.

This chapter focuses on biological profiles of acaricides that have been commercialized at the end of the 20<sup>th</sup> and beginning of the 21<sup>st</sup> century, acaricide resistance in phytophagous mites, bioactive products of natural origin as alternatives to synthetic acaricides, compatibility of acaricides with the biological control agents, and other current issues related to acaricide uses in modern crop protection.

## 2. Summary of acaricide development

As already remarked, phytophagous mites became important pests of cultivated plants in the mid-20<sup>th</sup> century, during the „golden age of insecticide discovery“ (Casida & Quistad, 1998) that was marked by intensive use of **organochlorines**, **organophosphates** and **carbamates**, broad spectrum insecticides which, as it was later discovered, included many acaricidal compounds. Those were the neuroactive compounds which disrupt the transmission of impulses between nerve cells of an insect by blocking the action of the enzyme acetylcholinesterase (organophosphates, carbamates) or interfering with ion channels in the nerve membrane (organochlorines) (Ishaaya, 2001). Figure 1 shows chlorpyrifos, probably the most common commercialized organophosphate today, carbaryl, the first synthesized carbamate, and endosulfan, one of the rare organochlorine compounds still in use.

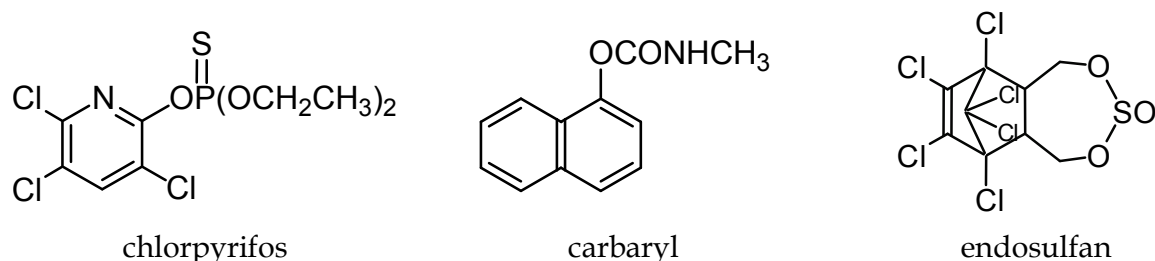
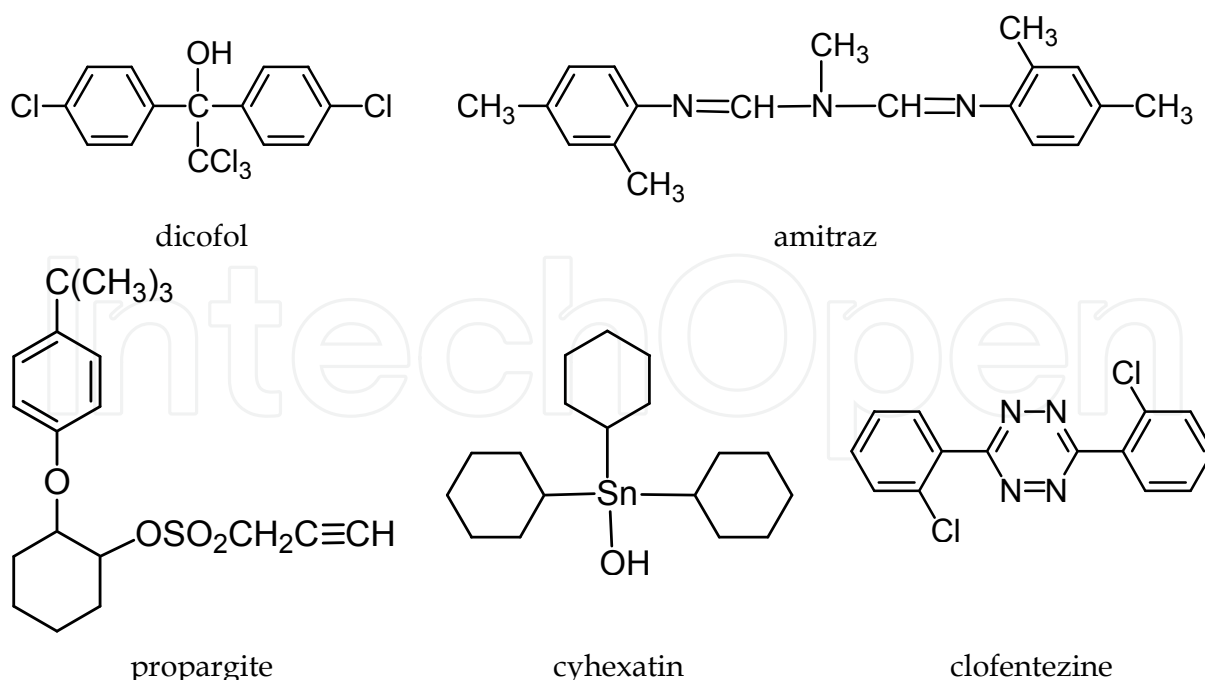


Fig. 1. Neuroactive insecticides with acaricidal activity: chlorpyrifos (organophosphate), carbaryl (carbamate) and endosulfan (organochlorine)

The first serious and widespread spider mite outbreaks following applications of neuroactive insecticides, observed at the end of 1940s and beginning of 1950s, initiated the research and development of specific acaricides. These compounds, exclusively or primarily effective against mites, were gradually taking over the organochlorines, organophosphates and carbamates. **Bridged diphenyls** (bromopropylate, chloropropylate, chlorobenzilate, chlorfenethol, dicofol, tetradifon), the first specific acaricides, established themselves on the market in the 1950s. During the 1960s and early 1970s, the second generation of structurally rather different specific acaricides emerged, the most important of which were **propargite**, **organotin**s (cyhexatin, fenbutatin-oxide) and **formamidines** (amitraz, chlordimeform). Most of first and second generation acaricides are not used any longer. Specific acaricides of the third generation are represented by **mite growth inhibitors** (clofentezine, hexythiazox), commercialized in the first half of the 1980s (Fig. 2) In addition to specific acaricides, several structurally diverse synthetic acarofungicides (dinocap, dinobuton, chinomethionate, dichlofluanid) were introduced; on the other hand, the use of sulfur products (that had been exploited as acarofungicides since 19<sup>th</sup> century) was largely displaced by novel synthetic compounds.

Introduction of specific acaricides reduced the adverse impact on beneficial insects (predators of insect and mite pests, pollinators) to the minimum; at the same time, many specific acaricides proved to be selective, i.e. less toxic to predaceous mites than phytophagous mites. These acaricides effectively control populations of phytophagous mites resistant to neuroactive compounds, since they are compounds having different biochemical modes of action, with targets mostly being outside the nervous system (March, 1976; Knowles, 1976, 1997; Ishaaya, 2001; Krämer & Schirmer, 2007). Moreover, specific acaricides are far more safer for humans, non-target organisms and the environment in





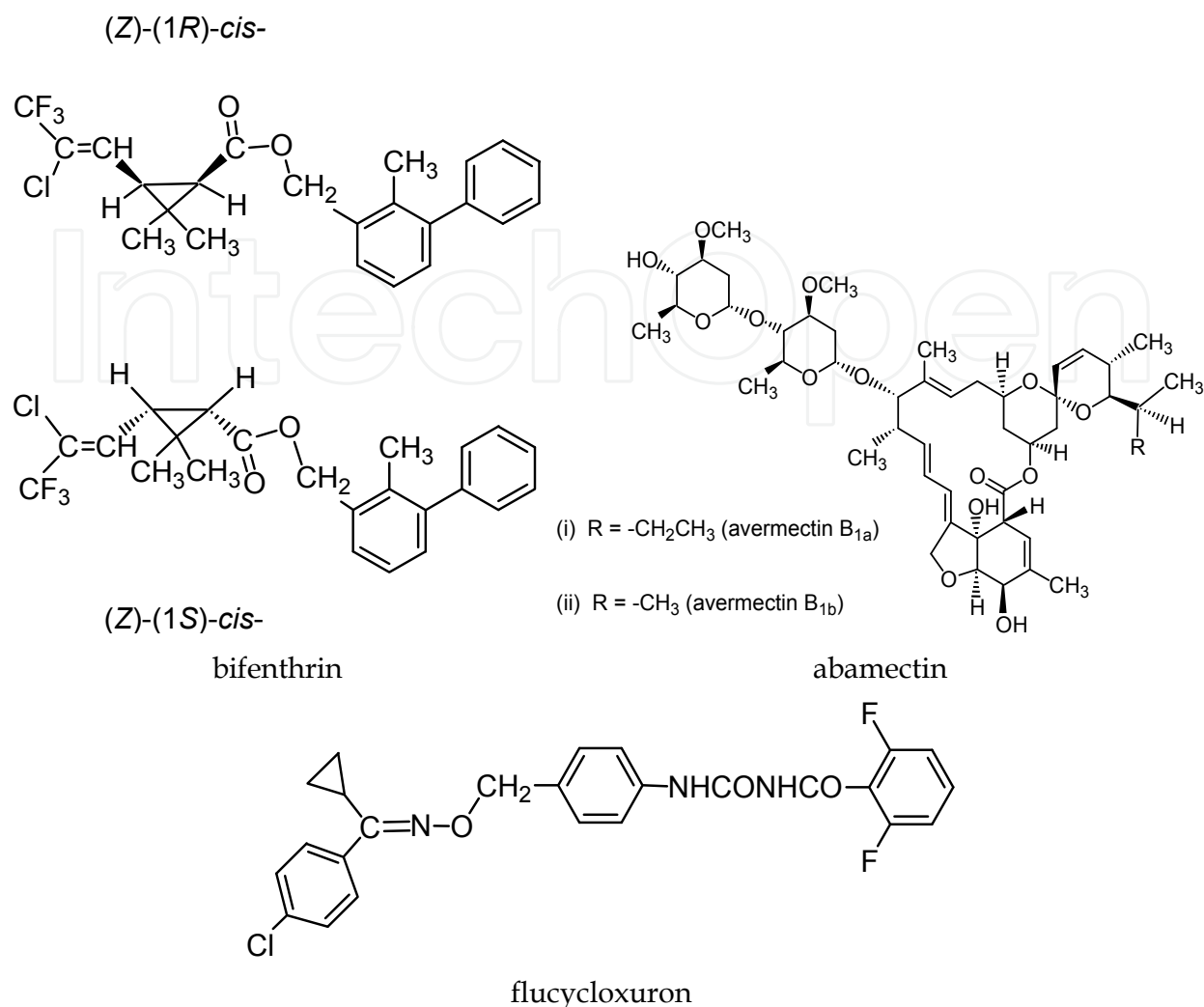


Fig. 3. Structural formulas of bifenthrin (pyrethroid), flucycloxuron (benzoylurea) and abamectin (natural product)

eco-toxicological criteria for market circulation of the existing pesticides and registration of the new ones imposed by the regulatory agencies and issues (Casida & Quistad, 1998; Dekeyser, 2005). In the USA, the passage of the Food Quality Protection Act (FQPA) of 1996 brought about significant changes in the way in which pesticides are registered by the U.S. EPA (Environmental Protection Agency). Besides re-evaluation of registered pesticides, priority in registration program has been given to "reduced-risk *pesticides*", i.e. pesticides with reduced risk to human health, non-target organisms and environment as a replacement for older and potentially riskier chemicals. The list of reduced-risk pesticides includes several new acaricides and insecto-acaricides (EPA, 2009, 2010). In the European Union, implementation of Directive 91/414 that requires science-based assessment of pesticide risk to human health and the environment, has seriously impacted the EU acaricide portfolio. Nevertheless, new Regulation (EC) 1107/2009, revises the Directive and introduces hazard-based cut-off criteria, thus increasing the safety level (Balderacchi & Trevisan, 2010; van Leeuwen et al., 2010). When looking at the acaricides, from 103 substances, only 26 are currently included in a 'positive' list of compounds (Annex I) and the status of another four is pending (EU, 2010).

### 3. New synthetic acaricides

#### 3.1 Acaricides acting on respiration targets

Similar to nervous system of insects, nervous system of mites has also long been the target for most chemicals used for their control (Casida & Quistad, 1998). The situation has somewhat changed during the last two decades due to commercialization of large number of acaricidal compounds acting on mitochondrial respiration process, that produces most of the energy in cells. This process includes two coupled parts: mitochondrial electron transport (MET) and oxidative phosphorylation. Although some of the older acaricides were known to inhibit respiration, the real exploitation of this target started no sooner than after the 1990s, with the prospects for expanding and developing new, more effective and safer products (Dekeyser, 2005; Lümme, 2007; Krämer & Schirmer, 2007).

Throughout the mitochondrial electron transport chain there are various potential sites for inhibition, but only three have been used so far as target sites of acaricidal activity, at transmembrane enzyme complexes. In the period 1991-93, four compounds from different chemical classes were successively commercialized: **fenpyroximate** (pyrazole), **pyridaben** (pyridazinone), **fenazaquin** (quinazoline) and **tebufenpyrad** (pyrazolecarboxamide) (Fig. 4), whose mode of action was inhibition of MET at complex I. These compounds, also known as METI acaricides, quickly gained the popularity worldwide owing to the high efficacy against both tetranychid and eriophyoid mites, quick knockdown effect and long-lasting impact. In addition, these substances have low to moderate mammalian toxicity and short to moderate environmental persistence (Dekeyser, 2005; Krämer & Schirmer, 2007, van Leewen et al., 2010). Fenpyroximate and tebufenpyrad are included in Annex I, while fenazaquin and pyridaben applications have been resubmitted for inclusion (EU, 2010). Fenpyroximate is also on the list of reduced risk and organophosphorus alternative pesticides (EPA, 2009). Complex I inhibitors also include **pyrimidifen** (pyrimidinamine), commercialized in 1995, as well as insecto-acaricide **tolfenpyrad**, another pyrazolecarboxamide, commercialized in 2002, and **flufenimer**, more recent derivative of pyrimidifen (Krämer & Schirmer, 2007).

The only known complex II inhibitor is the recently introduced insecto-acaricide **cyenopyrafen**, a compound from the acrylonitrile class of chemistry (Lümme, 2007). Complex III inhibition is mode of action of acequinocyl, fluacrypyrim and bifenazate. **Acequinocyl**, a naphthoquinone compound (Fig. 5) commercialized in 1999, is a pro-acaricide which is bioactivated via deacetylation. It is effective against all stages of spider mites, with low mammalian toxicity and short environmental persistence (Dekeyser, 2005). It is included in the EPA list of reduced risk pesticides, while the decision on its status under Directive 91/414 is pending (EPA, 2009; EU, 2010). **Bifenazate**, a carbazate compound (Fig. 5) is highly effective against immatures and adults of spider mites, with rapid knockdown effect (Ochiai et al., 2007). Although it was first considered to be a neurotoxin, more recent experimental results indicate complex III as target site (van Nieuwenhuysen et al., 2009). Bifenazate is a pro-acaricide which is bioactivated via hydrolysis of ester bonds, so the organophosphorous compounds, as inhibitors of esterase hydrolytic activity, can antagonize the toxicity of this acaricide (van Leeuwen et al., 2007). Bifenazate, introduced in 1999, is a compound of low mammalian toxicity and short environmental persistence; it is classified as a reduced risk and organophosphate alternative pesticide, and it is included in Annex I (Dekeyser, 2005; EPA, 2009; EU, 2010). **Fluacrypyrim**, introduced in 2002, shows acaricidal effect against all stages of tetranychids. This is the first strobilurin not commercialized as a fungicide (Dekeyser, 2005; Krämer & Schirmer, 2007), and more compounds with acaricidal effect from this group are anticipated (Li et al., 2010).

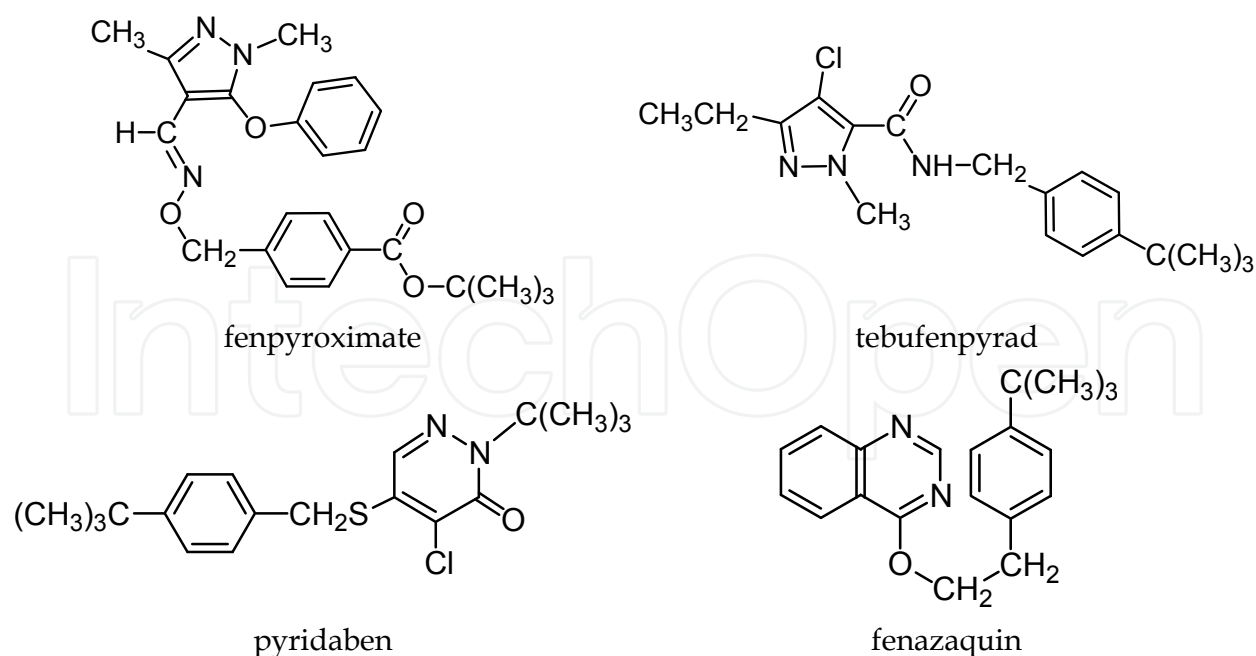


Fig. 4. Structural formulas of acaricides acting on respiration targets: METI acaricides

Insecto-acaricide **diafenthiuron**, a novel thiourea compound (Fig. 5) launched in 1991, is the only modern representative of compounds that disrupt oxidative phosphorylation by inhibition of the mitochondrial ATP synthase, an enzyme with essential role in cellular bioenergetics (this mode of action has been recognized in propargites, tetradifons and organotin compounds). Diafenthiuron is a pro-acaricide, its carbodiimide metabolite inhibits the enzyme. It is effective against motile stages of spider mites and also provides good eriophyoid control. Diafenthiuron has low mammalian toxicity and short environmental persistence (Krämer & Schirmer, 2007; van Leewen et al., 2010).

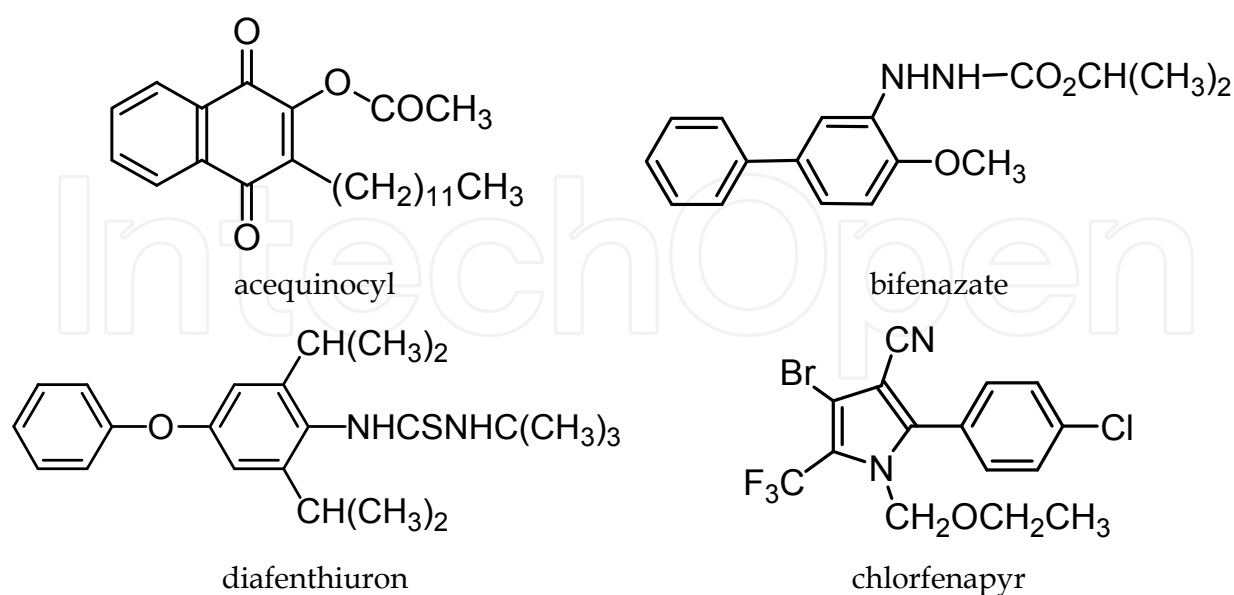


Fig. 5. Structural formulas of acaricides acting on respiration targets: complex III inhibitors (acequinocyl, bifentazate) and inhibitors of oxidative phosphorylation (diafenthiuron, chlorfenapyr)



Another insecto-acaricide, **chlorfenapyr**, a pyrrole compound (Fig. 5) commercialized in 1995, at biochemical level acts as uncoupler of oxidative phosphorylation via disruption of the proton gradient. Chlorfenapyr is effective against all stages of spider mites and eriophyoid mites. This compound is a pro-acaricide activated by N-dealkylation. Chlorfenapyr is a compound of moderate mammalian toxicity, but long environmental persistence (Krämer & Schirmer, 2007; Van Leeuwen et al., 2010). It is included in the EPA list as an alternative to organophosphorus compounds (EPA, 2009).

### 3.2 Acaricides acting on growth and development targets

Another direction in research and development of synthetic acaricides is directed towards compounds affecting developmental processes. **Etoxazole**, a oxazoline compound (Fig. 6), is acaricide highly effective against eggs and immatures of spider mites, non-toxic to adults, but it considerably reduces fertility of treated females (Kim & Yoo, 2002; Dekeyser, 2005). This acaricide, launched in 1998, is usually classified among mite growth inhibitors, together with clofentezine and hexythiazox, older acaricides that cause similar symptoms (Marčić, 2003; Krämer & Schirmer, 2007), but whose exact mode of action is unknown. On the other hand, Nauen & Smagghe (2006) provided experimental evidence that etoxazole acts as a chitin synthesis inhibitor similar to benzoylureas. Etoxazole is on the EPA list of reduced risk and organophosphorus alternative pesticides (EPA, 2009).

Discovery of **spirodiclofen** and **spiromesifen**, tetroneic acid derivatives (Fig. 6) launched in 2002-2004, broadened the biochemical diversity of acaricides by introducing a completely new mode of action. These compounds act as inhibitors of acetyl-CoA-carboxylase, a key enzyme in fatty acid biosynthesis. Spirodiclofen and spiromesifen are highly toxic to eggs and immatures of spider mites, while their effects on adult females are slower with fecundity and fertility reduction; their acaricidal effect is long-lasting and stable (Krämer &

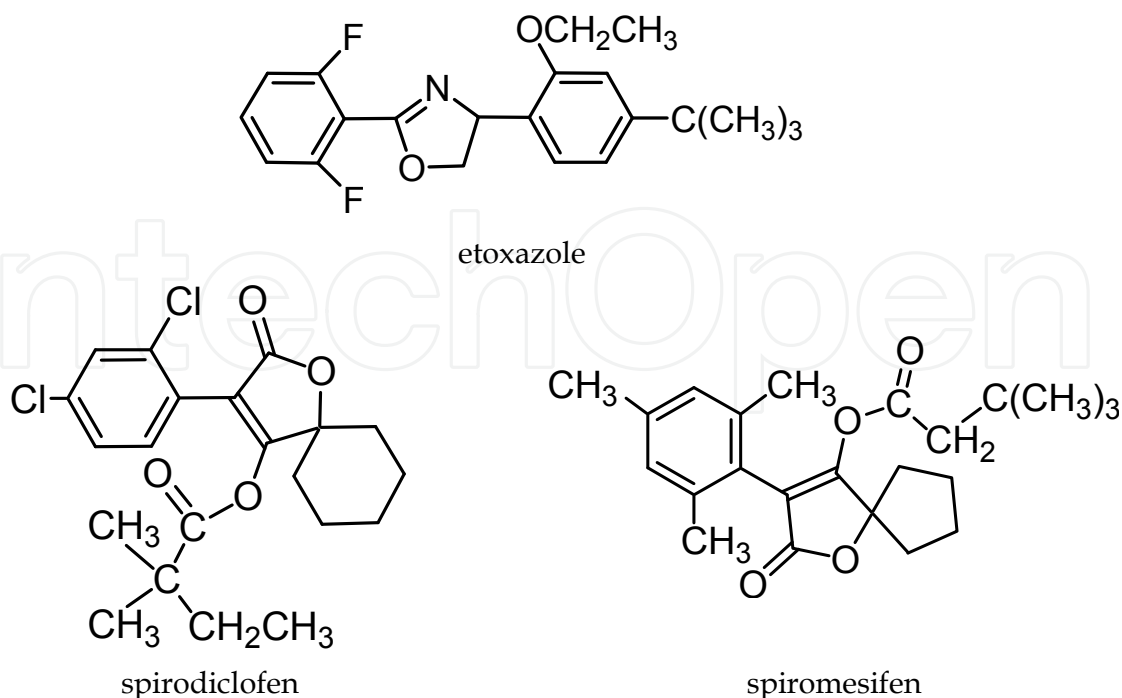


Fig. 6. Structural formulas of acaricides acting on growth and development

Schirmer, 2007; Marčić, 2007; Marčić et al., 2007; Van Pottelberge et al., 2009; Marčić et al., 2010). These two acaricides are the only new compounds used for control of eriophyoid mites as well (van Leeuwen et al., 2010). In addition to acaricidal effect, spiroadiclofen has also shown considerable insecticidal activity against eggs and larvae of pear psylla and scales (Krämer & Schirmer, 2007; Marčić et al., 2007), while spiromesifen provides effective control of whiteflies (Krämer & Schirmer, 2007; Kontsedalov et al., 2008). Both compounds have low mammalian toxicity and short environmental persistence. Spiroadiclofen is included in Annex I, and evaluation of spiromesifen is in progress (EU, 2010). **Spirotetramat**, a tetramic acid derivate recently introduced, belongs to inhibitors of acetyl-CoA-carboxylase. Although initially developed for control of whiteflies and aphids (Brück et al., 2009), the studies of its effects on *T. urticae* (Marčić et al., unpublished data) indicate that spirotetramat could potentially be an effective acaricide as well.

#### 4. Natural acaricides and other alternative solutions

The use of natural products for plant and crop protection dates back to times long before the introduction of synthetic pesticides which imposed themselves as the main means for suppression of harmful organisms. In recent times, the significance of natural pesticides is constantly growing, primarily in organic agriculture, but also in the framework of biorational pest control programs which insist on use of environmentally-friendly pesticides and exploitation of novel biochemical modes of action (Isman, 2006; Isman & Akhtar, 2007; Copping & Duke, 2007; Horowitz et al., 2009). Some of the natural products are substances that have significant acaricidal effect.

Probably the most studied botanical insecticide in the last twenty years is a triterpenoid **azadirachtin** (Fig. 7), the major active ingredient of extracts, oils and other products derived from the seeds of the Indian neem tree (*Azadirachta indica*). Neem-products are registered in over 40 countries as products for suppression of arthropod pests important in growing of fruit, vegetables and ornamental plants (Kleeberg, 2004; Milenković et al., 2005). The effects of azadirachtin on treated insects manifest slowly and they include complete or partial antifeedant response, delayed and/or disrupted moulting, inhibited reproduction (Copping & Duke, 2007; Isman & Akhtar, 2007). The studies on spider mites (Sundaram & Sloane, 1995; Mansour et al., 1997; Martinez-Villar et al., 2005) indicate that azadirachtin, in addition to being toxic to various development stages, acts as antifeedant, reduces fecundity and fertility and shortens the life span of adult insects. Beside on spider mites, azadirachtin also exhibits acaricidal effect on some acarid and tarsonemid mites (Collins, 2006; Venzon et al., 2008). Azadirachtin is considered to be non-toxic to mammals and is not expected to have any adverse effects on the environment (Copping & Duke, 2007); its Annex I application is resubmitted (EU, 2010). Many neem/azadirachtin-based products are approved for use in organic crop production (Zehnder et al., 2007; EU, 2008; Dayan et al., 2009).

Products isolated from soil actinomycetes are an important source for deriving natural insecticides and acaricides. In early 1990s, several years after introduction of abamectin, another fermentation product, **milbemectin**, was commercialized. Milbemectin is a mixture of milbemycin A<sub>3</sub> and milbemycin A<sub>4</sub>, natural products isolated from the fermentations of *Streptomyces hygroscopicus* subsp. *aureolacrimosus* (Fig. 7). Milbemectin is a neuroactive acaricide (chloride channel activator), effective against tetranychid and eriophyoid mites, relatively safe compound owing to the rapid uptake into treated plants combined with fast degradation of surface residues (Copping & Duke, 2007; Krämer & Schirmer, 2007). Like abamectin, milbemectin is also included in Annex I (EU, 2010).

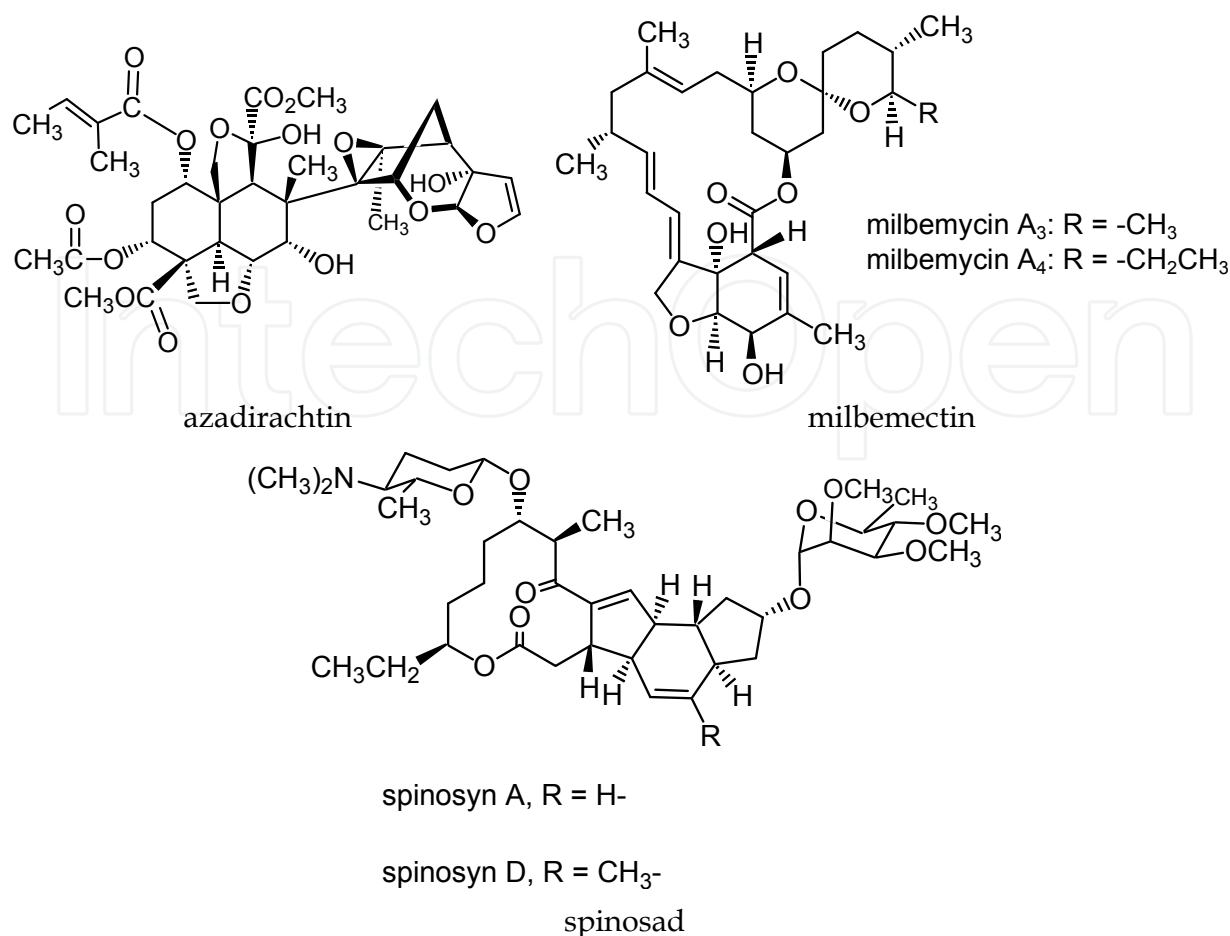


Fig. 7. Natural products with considerable acaricidal activity

The more recent example is **spinosad**, a mixture of spinosyn A and spinosyn D, secondary metabolites of *Saccharopolyspora spinosa*, (Fig. 7), introduced in 1997 as neuroactive insecticide, nicotinic acetylcholine receptor agonist (Copping & Duke, 2007; Krämer & Schirmer, 2007). This insecticide exerts significant acaricidal effect. Van Leeuwen et al. (2005) found out that the residual toxicity of spinosad to female *T. urticae* is equal to the level of toxicity resulting from application of dicofol, bromopropylate or fenbutatin oxide, while Villanueva & Walgenbach (2006) demonstrated that spinosad affects larvae and adults of this tetranychid, but relatively slowly, with the assumption that negative results of the previous testing of acaricidal properties were based on experiments that did not provide enough time for response. Spinosad shows systemic acaricidal effect, if used for substrate watering, such as rockwool, where the absorption level is reduced to the minimum (Van Leeuwen et al. (2005). This is a compound of very low mammalian toxicity and highly favourable environmental profile; it is included in the EPA list of organophosphorus alternative pesticides, and also in Annex I (EPA, 2009; EU, 2010). Spinosad is approved for use as an organic insecticide (EU, 2008; Dayan et al., 2009).

**Essential oils**, secondary metabolites abundant in some aromatic plants from families Lamiaceae, Apiaceae, Rutaceae, Myrtaceae and others, have been suggested as alternative sources for pest control products. Predominant bioactive ingredients of essential oils are monoterpenes and sesquiterpenes. Besides exerting acute toxicity to insects and mites, essential oils show sublethal effect as repellents, antifeedants and reproduction inhibitors. Lethal and sublethal effects of essential oils are the consequence of direct contact and/or

uptake of gas-phase via respiratory system. Insect octopaminergic nervous system is considered to be the target site of action of some essential oil constituents, but this may not be the case considering their acaricidal activity. Moreover, there is a possibility that essential oils, as complex mixtures, act at multiple target sites (Isman, 2006; Miresmailli et al., 2006; Isman & Akhtar, 2007; Shaaya & Rafaeli, 2007). Essential oils extracted from caraway seeds, eucalyptus, mint, rosemary, basil, oregano, thyme, and other plants have shown a significant acaricidal activity (Aslan et al., 2004; Choi et al., 2004; Miresmailli et al., 2006). These oils could be useful as fumigants in the control of phytophagous mites in greenhouses; however, for improving acaricidal activity, their commercial formulations need to be developed (Choi et al., 2004; Han et al., 2010). Essential oils are mostly nontoxic to mammals; being volatile products, they have limited environmental persistence (Isman, 2006). Rosemary oil, thyme oil and some other essential oils are available for pest control in organic farming (Dayan et al., 2009).

**Petroleum oils** have been used for more than a century to control a wide range of crop pests, including spider mites. Because of their high phytotoxicity, the use of petroleum oils was limited to dormant or delayed dormant application against overwintering pest stages, to avoid injury to green plant tissue. Advances in petroleum chemistry considerably reduced phytotoxicity in newer, highly-refined petroleum-derived spray oils (PDSO), which are recognized today as an important alternative to synthetic pesticides. PDSO are environmentally-friendly products with negligible impact on human health and the environment. The most widely accepted theory on their mode of action is that PDSO primarily act physically by blocking the spiracles in insects (or the stigmata in mites) and thus causing suffocation, but it can not be presumed as the only mode of action (Taverner, 2002). At least some modern oils cause a range of cellular disruption leading to rapid insect death (Najar-Rodriguez et al., 2008). PDSO are highly effective against spider mites and eriophyoid mites in various field and greenhouse crops (Agnello et al., 1994; Nicetic et al., 2001; Marčić et al., 2009; Chueca et al., 2010). Beside mineral, **plant oils** proved to be effective acaricides as well, such as cottonseed oil (Rock & Crabtree, 1987) soybean oil (Lancaster et al., 2002; Moran et al., 2003) and rapeseed oil (Kiss et al., 1996; Marčić et al., 2009). PDSO and plant spray oils are considered compatible with organic farming (Zehnder et al., 2007; EU, 2008). Rapeseed oil is included in Annex I (EU, 2010).

Numerous studies indicate that entomopathogenic fungi, especially ascomycetes, can play an important role in regulation of harmful arthropod populations if used in biological control (Hajek & Delalibera, 2010), or applied as **mycoinsecticides** and/or **mycoacaricides** (Maniania et al., 2008; Jackson et al., 2010). Among the entomopathogenic fungi, the most potent pathogens of tetranychids and other pest mite species are *Beauveria bassiana*, *Hirsutella thompsonii*, *Lecanicillium* sp., *Metharizium anisopliae*, *Isaria fumosorosea*, *Neozygites floricola* (Chandler et al., 2000; Maniania et al., 2008), whose conidia and blastospores are used for formulation of fungal-based biopesticides. At the beginning of the 1980s, only one mycoacaricide was available (Mycar), formulated from conidia of *H. thompsonii* and intended for suppression of citrus rust mite. Quarter of century later, there are some 30 commercial products acting against tetranychid, eriophyoid, and tarsonemid mites, mostly formulated as wettable powder or oil dispersion, and one third of which is made from conidia of *B. bassiana* (Faria & Wraight, 2007).

## 5. Acaricide resistance in phytophagous mites

As a result of exceptional intrinsic potential of mites for rapid development of resistance (Cranham & Helle, 1985; Croft & van de Baan, 1988; van Leeuwen et al., 2009) and often not



so rational actions of humans, the acaricide resistance in mites, in particular the species from Tetranychidae family, has become a global phenomenon. Arthropod Pesticide Resistance Database (APRD) - managed by scientist from Michigan State University and supported by Insecticide Resistance Action Committee (IRAC), a specialist technical group of the industry association CropLife – contains published data on resistance in insects and mites important for agriculture, veterinary medicine and public health, from 1914 to date (Whalon et al., 2008, 2010). This database, which involves a large number of scientists and experts from around the world who work on its administration and upgrading, is useful for comprehension of acaricide resistance in mites on a global level.

In the mid-2010, APRD contained 9394 reports on resistance developed in 572 species of arthropods, of which 1130 reports refer to 82 species from Acari subclass. Out of this number, 745 reports concern 39 species belonging to four families of phytophagous mites: Tetranychidae, Acaridae, Eriophyidae and Tenuipalpidae. Approximately 93% of reports deal with the resistance of spider mites, with two predominant species two-spotted spider mite, *Tetranychus urticae* (53% of spider mite reports) and European red mite, *Panonychus ulmi* (26% of spider mite reports) (Tab. 1). The authors of the APRD created the list of the "top 20" resistant arthropod pests in the world, ranked by number of compounds with reported resistance. On this list, *T. urticae* and *P. ulmi* rank first and ninth, respectively, by data for 92 and 42 compounds for which the information about resistant populations exist (Whalon et al., 2008, 2010).

For both species, the majority of reports refer to resistance to organophosphates documented during the 1950s, 1960s, and 1970s. Together with carbamates, organophosphate compounds account nowadays for more than 35% of global insecticide market, so that the reports on resistant tetranychid populations/strains are still coming (Herron et al., 1998; Stumpf et al., 2001; Tsakaragkou et al., 2002; Kumral et al., 2009). The important part of the APRD database concerns pyrethroids resistance in tetranychids. Today, this class of compounds accounts for 20% of the market, but the increasing number of cases of resistance to bifenthrin and other pyrethroids has been registered in the recent past (Herron et al., 2001; Ay and Gürkan, 2005; Kumral et al., 2009; Tsakaragkou et al., 2009). As for other specific acaricides and insecto-acaricides, there is practically no active substance without documented cases of resistance, but there is an obvious difference in the scope of phenomenon between certain acaricides or groups of acaricides. For instance, global popularity of METI-acaricides contributed to relatively fast development of resistant spider mite populations in Japan, South Korea, Australia, Brazil, California and some European countries. On the other hand, there are only few reports on resistance to fenbutatin-oxide and other organotin compounds which have been used for four decades now (Stumpf & Nauen, 2001; Auger et al., 2004; van Leeuwen et al., 2009; Stavrinides et al., 2010).

Another phytophagous mite on the list of „top 20“, bulb mite *Rhizoglyphus robini* (Acaridae), ranks 19<sup>th</sup> with 22 reports on resistance to almost exclusively organophosphate compounds. Cases of resistance to organophosphates are also registered for other species of acarids listed in the APRD. Among eriophyoid mites, resistance in citrus rust mite, *Phyllocoptruta oleivora*, to dicofol has been best documented and most studied (Omoto et al., 1994); other cases of resistance in Eriophyoidea also refer to organophosphorous compounds.

In addition to comprehensive documenting of acaricidal resistance in mites, the factors affecting this phenomenon of microevolution were also studied, as well as its physiological, biochemical and genetic mechanisms. The results of these studies were summarized by Cranham & Helle (1985), Croft & van de Baan (1988), Messing & Croft (1996), Knowles



Mite species	No. of cases	No. of compounds
<i>Tetranychus urticae</i>	367	92
<i>Panonychus ulmi</i>	181	42
<i>Panonychus citri</i>	26	20
<i>Tetranychus cinnabarinus</i>	26	16
<i>Tetranychus mcdanieli</i>	19	13
<i>Tetranychus kanzawai</i>	12	12
<i>Tetranychus viennensis</i>	7	7
<i>Tetranychus atlanticus</i>	7	5
<i>Tetranychus pacificus</i>	7	5
<i>Oligonychus pratensis</i>	6	6
<i>Tetranychus turkestani</i>	5	5
<i>Tetranychus hydrangaea</i>	5	4
<i>Tetranychus arabicus</i>	3	3
<i>Tetranychus crataegi</i>	3	3
<i>Tetranychus desertorum</i>	3	3
<i>Tetranychus ludeni</i>	3	3
<i>Tetranychus bimaculatus</i>	2	2
<i>Tetranychus cucurbitacearum</i>	2	2
<i>Tetranychus schoenoi</i>	2	2
<i>Tetranychus tumidus</i>	2	2
<i>Eotetranychus hicoriae</i>	1	1
<i>Tetranychus althaeae</i>	1	1
<i>Tetranychus canadensis</i>	1	1
Tetranychidae	691	
<i>Rhizoglyphus robini</i>	22	22
<i>Rhizoglyphus echinopus</i>	6	5
<i>Acarus siro</i>	4	3
<i>Acarus chaetoxysilus</i>	2	2
<i>Acarus farris</i>	1	1
<i>Tyrophagus palmarum</i>	1	1
<i>Tyrophagus putrescentiae</i>	1	1
Acaridae	37	
<i>Phyllocoptruta oleivora</i>	3	2
<i>Aculus cornutus</i>	3	3
<i>Aculus pelekassi</i>	3	3
<i>Aculus fockeui</i>	1	1
<i>Aculus lycopersici</i>	1	1
<i>Aculus malivagrans</i>	1	1
<i>Aculus schlechtendali</i>	1	1
Eriophyidae	13	
<i>Brevipalpus chilensis</i>	3	3
<i>Brevipalpus phoenicis</i>	1	1
Tenuipalpidae	4	
Total	745	

Tab. 1. Reported cases of acaricide resistance in phytophagous mites (Whalon et al., 2010)

(1997), van Leeuwen et al. (2009), and the largest number of data refers to populations/strains of *T. urticae*. As in other arthropods, the resistance in mites is caused by a less sensitive target site (**target site resistance**) and/or enhanced detoxification (**metabolic resistance**). The insensitivity of acetylcholinesterase is the most common type of organophosphorous resistance in *T. urticae* (Cranham & Helle, 1985; Stumpf et al., 2001; Tsagkarakou et al., 2002; van Leeuwen et al., 2009; Khajehali et al., 2010; Kwon et al., 2010a). Metabolic resistance mediated by carboxylesterases was found in majority of cases of resistance development to pyrethroids in this species (Ay & Gürkan, 2005; van Leeuwen et al., 2005b, van Leeuwen & Tirry, 2007), while the oxidative metabolism appears to play a major role in resistance to METI-acaricides (Stumpf & Nauen, 2001; Kim et al., 2004, 2006; van Pottelberge et al., 2009).

The results of numerous conventional genetic studies indicate that in most cases single major gene controls inheritance of resistance in spider mites (Cranham & Helle, 1985; van Leeuwen et al., 2009). Although the monogenic and dominant resistance has been expected due to intense selection pressure to which the populations under the open field or greenhouse conditions are exposed (Roush & McKenzie, 1987), some major exceptions occur, such as the monogenic-recessive resistance to dicofol (Rizzieri et al., 1988), propargite (Keena & Granett, 1990), pyridaben (Goka, 1998) and etoxazole (Uesugi et al., 2002), and poligenic resistance to cyhexatin (Mizutani et al., 1988). Lately, several studies dealing with molecular basis of the target site resistance to pyrethroids (Tsagkarakou et al., 2009; Kwon et al., 2010b), organophosphates (Khajehali et al., 2010; Kwon et al., 2010a) and bifenazate (van Leeuwen et al., 2008, van Nieuwenhuysse et al., 2009) have been published. Especially interesting discovery is that the bifenazate resistance in *T. urticae* is inherited only maternally, which is the first occurrence of non-Mendelian inheritance since the beginning of genetic studies on pesticide resistance in arthropods (van Leeuwen et al., 2008, van Nieuwenhuysse et al., 2009).

Biological, biochemical and genetic characterization of resistance is one of the essential elements in defining the strategy for management of acaricide resistance in phytophagous mites. An effective acaricide resistance management program could be based on general resistance management principles endorsed by IRAC (Krämer & Schirmer, 2007). The key recommendation is reduction of the selection for resistance which is possible to attain if there were available as many as possible acaricides with different modes of action. The history of resistance in *T. urticae* best illustrates the importance of the above: the first resistant populations can emerge as soon as after two or three years from the start of a new acaricide application, causing an obvious pest control failure (Cranham & Helle, 1985; Knowles, 1997; van Leeuwen et al., 2009).

In the European Union, the implementation of Directive 91/414 reduced the EU acaricide portfolio by more than 70% (EU, 2010; van Leeuwen et al., 2010). On the other hand, it is Directive 91/414 that requires pesticide registrants to address the risk of resistance development as part of dossiers submitted for EU registration (Thompson et al., 2008). In „Declaration of Ljubljana“ (Bielza et al., 2008) a group of leading resistance management experts expressed strong concern that further loss of active ingredients resulting from the implementation of Directive 91/414 (and its revision) could endanger the sustainability of European farming, increasing the risk of developing resistance to the relatively few remaining substances. The scientists concluded that the resistance management requires

access to a diversity of chemistries with different modes of action. Considering the fact that every year only few new active substances are registered in the EU, it is clear that the pesticide industry is unable to offer enough replacements for the products which are being withdrawn from the market (Thompson et al., 2008).

## 6. Acaricides and integrated control of phytophagous mites

Biological control of phytophagous mites by predatory mites (Phytoseiidae) and other predators proved to be a successful alternative to conventional chemical control, especially on greenhouse crops (Gerson & Weintraub, 2007). In spite of undoubtful advantages, biological control includes significant limitations as well (Gerson et al., 2003), which makes the use of acaricides still indispensable. In modern crop protection, these acaricides should be biorational compounds: highly effective against mite pests and relatively safe to their predators (i.e. selective), with low risk to human health and the environment. Biorational acaricides are important element of integrated control of phytophagous mites which is based on combination of chemical, biological and other control measures.

Therefore, it is very important to study the effects of acaricides and other pesticides on phytoseiid mites, other predatory mites and insect predators of phytophagous mites. Predators come into contact with pesticides if treated with them directly or exposed to their residues, if they feed on contaminated prey or pollen. Beside lethal effects (mortality), pesticides also cause a variety of sublethal effects, by changing the biological parameters and/or behaviour of survivors (Blümel et al., 1999; Desneux et al., 2007). International Organization for Biological Control/Western Palearctic Regional Section (IOBC/WPRS) offered one of the most comprehensive programs to test lethal and sublethal side-effects of pesticides on beneficial organism, which comprise laboratory, semi-field and field trials. IOBC/WPRS working group „Pesticides and beneficial organisms“ organized and carried out several joint testing programs for most of the predators, parazitoids and other beneficial organisms, including phytoseiid mites (Blümel et al., 1999; Blümel & Hausdorf, 2002). However, some methodological solutions within the IOBC procedures (way of exposure, choice of doses/concentrations, evaluation criteria) have been criticized as insufficiently realistic (Bakker & Jacas, 1995; Amano & Haseeb, 2001). In order to acquire an in-depth knowledge on sublethal effects of pesticides on biological control agents, the population level-toxicity approach was proposed; it is based on creation of life tables and calculation of population growth parameters, and/or projection of population growth rate based on matrix model (Stark & Banks, 2003; Stark et al., 2007).

The most frequently encountered on the lists of non-selective active substances are organochlorines, organophosphates, carbamates, pyrethroids and other broad-spectrum insecto-acaricides, which are *per definitionem* toxic to large number of insect and mite species, including Phytoseiidae and majority of other arthropods, predators of phytophagous mites (Croft & Brown, 1975; Knowles, 1997; Blümel et al., 1999; Gerson et al., 2003). On the other side, abamectin and milbemectin, which are also broad-spectrum insecto-acaricides, are considered safe to beneficial arthropods under field conditions due to their short environmental persistence, rapid uptake into treated plants and fast degradation of surface residues (Krämer & Schirmer, 2007). Although beneficials may be killed when treated directly by spray oils or exposed to the vapor phase of essential oils, their short-term residual activity does not severely affect populations of phytoseiid mites and other predators (Chueca et al., 2010; Han et al., 2010).

It should be noted that certain fungicides (benomyl, dithiocarbamates) are partly harmful to predatory mites (Blümel et al., 1999; Gerson et al., 2003; Alston & Thomson, 2004). Sulfur, acaro-fungicide approved in organic farming (Zehnder et al., 2007; EU, 2008; Milenkovic et al. 2010) have been identified as disruptive to integrated mite control (Beers et al., 2009). Also, there are records of adverse effects of neonicotinoids (new class of neuroactive insecticides which is in great expansion in the last two decades), on survival and/or fecundity (James, 2003; Duso et al., 2008), predator activity (Poletti et al., 2007) and population growth (Stavrinides & Mills, 2009) of phytoseiid mites.

Specific acaricides are considered harmless to majority of predatory insects, while their toxicity to various development stages of the same mite species, and to different mite species, varies to a certain extent. From the standpoint of selectivity, it is essential to be aware of the comparative toxicity of acaricides to phytophagous mites and predatory mites (Knowles, 1997). Compounds, such as organotins, mite growth inhibitors and regulators, acequinocyl, diafenthiuron, some METI acaricides, bifenazate, spiroadiclofen, spiromesifen, are usually graded as selective acaricides, much more toxic to phytophagous mites than to phytoseiid and other predatory mites (Blümel et al., 1999; Knowles, 1976; Dekeyser, 2005; Krämer & Schirmer, 2007). Spinosad and azadirachtin appear to be compatible with predatory mites (Spollen & Isman, 1996; Williams et al., 2003; Raguraman et al., 2004).

Both positive and negative evaluation results are based on smaller or larger number of experimental data, but they should not be taken as general and final conclusions on (non)selectivity. Besides expected intrinsic differences among predatory species in susceptibility to the same pesticide, the literature provides different, and sometimes even contrasting results on compatibility for the same active substance and the same predatory species, due to different test procedures (applied doses/concentrations, way of treatment and exposure of test organisms, observed parameters, laboratory or field experiments); on the other hand, the results obtained by standardized methods are affected by the product formulation type, origin of test organism (autochthonous population or commercialized strain) and other factors (Blümel et al., 1993; Duso et al., 2008).

Physiological selectivity, i.e. reduced susceptibility due to pesticide metabolism is the most desired testing result of pesticide effects to phytophagous mite predators. But, the non-selective compounds can be made safer for use by special application technology (Blümel et al., 1999; van Leeuwen et al., 2005a), by reducing the doses/concentrations (Rhodes et al., 2006), by releasing the predators so that they would be exposed to older residues (Lilly & Campbell, 1999), by using the strain of predators with developed resistance to acaricides and other pesticides (Sato et al., 2007).

Application of selective acaricides (synthetic or natural) with releases of commercialized strains of phytoseiid mites and other predators is a sustainable alternative to an approach based on chemical measures only (Lilly & Campbell, 1999; Rhodes et al., 2006; Sato et al., 2007). According to Kogan (1998), a pest control program reaches the level at which it can be qualified as an integrated pest management (IPM) program only when biorational pesticides (acaricides) and release of predators are integrated with other control tactics, preventive and remedial (crop rotation, host plant resistance, cultural practices, mechanical and physical control measures etc). Higher IPM levels entail transfer from species/population level integration (the control of single species or species complexes), via community level integration (multiple pest categories e.g. insects, mites, pathogens, weeds and their control) to ecosystem level integration (the control of multiple pest impacts within the context of the total cropping system).



World-wide, IPM has become the accepted model for crop protection over the past decades, but the adoption of IPM programs has been generally slow in both the developed and the developing countries (Peshin et al., 2009). In the European Union, Directive 91/414 encourages Member States to take the principles of IPM into account, but the implementation is voluntary (Freier & Boller, 2009). Success of IPM has often been measured by the reduction in pesticide usage, which is not necessarily a reliable indicator (Kogan, 1998). Transition from conventional pest control to IPM actually changes the role of pesticides (acaricides) in modern crop protection: within the principles of IPM, pesticides are applied highly rationally and in interaction with other control tactics.

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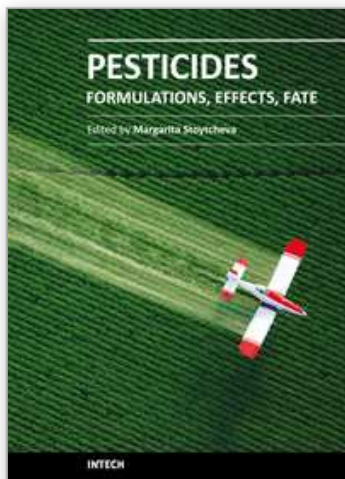
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### **Pesticides - Formulations, Effects, Fate**

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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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