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Review

Synthetic Insecticides – is There an Alternative?

Szymon Chowański*, Milena Kudlewska, Paweł Marciniak, Grzegorz Rosiński

Department of Animal Physiology and Development, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland

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Abstract

Synthetic insecticides are very efficient in insect control but can be harmful for the environment and health. They cause disturbances in ecosystem functioning, are toxic for a wide range of non-target organisms, and have a high tendency to accumulate in the environment. Because of this, some alternatives are being sought. A good solution seems to use biologically active peptides like peptide hormones, neurohormones, or neuromodulators to regulate major processes in insects: development, growth, reproduction, and metabolism. Peptides, such as trypsin modulating oostatic factor (TMOF), pheromone biosynthesis activating neuropeptides (PBANs), pyrokinins (PKs), sulfakinins (SKs), and allostatins (ASTs), as well as their analogues, have been extensively studied to produce pseudopeptides and peptidomimetics used by modern agriculture in contrast to chemical insecticides.

Keywords: synthetic insecticides, bioinsecticides, biologically active insect peptides

Introduction

One of the main problems in agricultural production is crop destruction by pests, mainly by insects [1, 2]. At present, a common way to control insect pests is the use of synthetic pesticides, but they have a negative influence on the natural environment [3]. Synthetic insecticides have a wide spectrum of activity against diverse groups of insects and cause almost complete removal of the pest from the crop area. However, the speed and efficiency of synthetic insecticides is only seemingly positive. There is no possibility to limit the action of these compounds only to crop areas. Significant parts of insecticides applied in different ways penetrates into the surrounding farmland ecosystems and acts destructively, not only on the invertebrates, but also on the vertebrates. Synthetic insecticides have a long half-life, which causes their retention in the environment for long periods of time, often several times exceeding the lifetime of different species' generations of animals. Furthermore, these compounds have a tendency to accumulate in different trophic levels of the food net [4, 5].

*e-mail: szyymon@amu.edu.pl

Considering the problems connected with using synthetic pesticides as a negative influence on the environment, insect resistance developing in insect pests, influence on human health, and socioeconomic costs, we can ask the question: Is there any alternative for these compounds? A new approach to control insect pests includes searching for compounds that specifically affect the physiological processes of insects and do not influence other groups of animals.

Bioinsecticides, a large group of substances derived from natural sources, such as animals, plants, bacteria, and certain minerals, seem to be an excellent alternative to synthetic insecticides. These compounds often have lower toxicity to non-target organisms than synthetic insecticides and they are effective at low concentrations and are readily biodegradable, which allows for avoiding the problems of environmental pollution [4]. This group include chitin synthesis inhibitors, hormone analogues (e.g. juvenile hormone, ecdysone), insect pheromones [6], and secondary plant metabolites, especially alkaloids [7, 8]. These compounds are characterized by a short period of half-life in the environment and rapid degradation into harmless compounds [9]. The need to create a safe and environmentally

friendly insecticides that eliminate only the target species has caused studies on the use of natural compounds as insecticide agents to be the most intensively developing stream of research.

Problems Connected with Using Synthetic Insecticides

Insect Resistance to Insecticide

One of the most serious problems associated with the use of synthetic insecticides is the development of insect resistance to these compounds. This process leads to the development of insect populations insensitive to most classes of insecticides.

Resistance to insecticides can be defined as the ability of an individual (and/or a population) to develop insensitivity to the action of toxic compounds in a significantly higher degree than in the basic population and the ability to pass this feature from one generation to the next. The development of resistance is caused by the occurrence of mutations in a population that becomes insensitive to the pesticide. During the influence of constantly acting as a selecting agent, allele conditioning resistance gradually increases its frequency in the population over time. After a specified time, the frequency of allele rises past critical value, which causes a given pesticide to become useless, because a significant part of the population does not show sensitivity to it [10-13]. The feature of resistance is not stable, particularly in populations with high heterozygosity. If the force action of a selecting agent weakens in the populations, the frequency of allele which determines resistance decreases [14]. Intensive use of insecticides is one of the strongest factors responsible for the rapid development of resistance in many species of insects [15].

The first well-documented case of insect resistance to insecticides was observed in 1946. It was resistance to dichlorodiphenyltrichloroethane (DDT) discovered in the housefly *Musca domestica* [11]. By 2009 there were more than 600 species of invertebrates resistant to at least one synthetic compound used in plant protection [16]. *Aphids* (*Aphidoidea*) are the group in which resistance develops the fastest. These insects are the record holders with regard to the number of insecticides to which they are insensitive (approximately 70 different synthetic compounds used for their control) [13].

It should be noted that the level of resistance to the same insecticides between the individual populations is significantly different and can reach up to 2,000 times. The rate of resistance development depends on several factors:

(i) rate of reproduction of insects
(ii) extent and rate of migration
(iii) host range
(iv) insecticide specificity
(v) time of action
(vi) time and number of insecticide applications [17].

The rapid development of resistance also is influenced by overlapping generations (mainly parental females generation with offspring), the presence of haploid and diploid individuals in the population and the r-type life strategy [18-20].

There are several mechanisms that enable insects to avoid toxic effects of insecticides, including:

(i) metabolic resistance
(ii) target-site resistance [21-25]
(iii) behavioral resistance
(iv) slow down the absorption of toxic compounds e.g. through appropriate modifications of the cuticle [18, 24, 26].

Metabolic resistance is the result of degradation of insecticides to completely non-toxic or less toxic products carried out by different enzymes. The major detoxification reactions are catalyzed by monooxygenases, carboxylesterases, and esterases.

Monooxygenases

Cytochrome P450-dependent monooxygenases are an important and diverse family of enzymes with the integrated hydrophobic heme molecule. These enzymes are involved in the metabolism of many endogenous (including hormones and lipids) and exogenous compounds. It causes the detoxification of the substrate and it takes place in mosquitoes, butterflies, houseflies, lice, and the cockroach *Blattella germanica* [26]. A huge variety of enzymes activities from this group is determined by the numerous isoforms, the wide range of substrates, and varying range of expression of genes encoding these proteins. There were many reports confirming increased monooxygenase activity in insects showing resistance to the insecticides, especially research with use the monooxygenases' inhibitors like piperonal butonide. In the presence of this inhibitor the resistance to insecticides was significantly reduced [24]. In most cases which demonstrated a correlation between increased activity of P-450-dependent monooxygenases and insect resistance to insecticides, there was observed increased activity of *cyp* genes belonging to the family *cyp6* [22].

Carboxylesterases

Carboxylesterases catalyze the hydrolysis of ester bonds. The share of carboxylesterases in resistance to insecticides is connected with a rapid hydrolysis of these compounds to inactive forms [25]. The high level of hydrolytic activity may be due to the higher activity of the enzyme [21, 27, 28], an increase in the amount of enzyme [13, 22], or a higher esterase affinity to substrates [27]. When the genes encoding carboxylesterases are overexpressed, these enzymes may be up to 0.4% of the total protein in body insect [29]. The increase in hydrolytic activity was observed in the case of resistance to organophosphorus compounds in *M. domestica* and *Lucilia cuprina* flies [27] and *Anisopteromalus calandrae* wasp [13].

Glutathione S-transferases

Glutathione S-transferases are present in all aerobic organisms. They are involved in detoxification of xenobi-

otics and endogenous compounds both in direct (O-dealkylation, O-dearylation, dehydrochlorination) and indirect way by catalysis of the secondary metabolites oxidized by cytochrome P-450-dependent monooxygenases [13] and detoxification products of lipids peroxidation generated during action of the insecticide [13, 26]. The main insect glutathione S-transferases responsible for insect resistance belong to Delta and Epsilon classes (these classes are found only in insects) [12, 30, 31]. The high activity of the enzymes is typically achieved by increasing the amount one or more enzymes isoforms or by amplification of the gene(s), or more often by the growth rate of transcription but without the quality change of individual enzymes [32].

The above-described examples of insect resistance were based on the metabolic mechanism, meaning the existence of enzymatic tools that effectively eliminate the toxic effects of the synthetic compound. The other effective mechanism of resistance to insecticides is so-called target site resistance, where the target of the insecticide action is changed. That can be enzymes (acetylocholinoeserase), receptors (γ-aminobutyric acid receptor), or ion channels (voltage gated sodium channel). The structural modifications by changes in the amino acid sequence cause the enzyme, receptor, or channel to become partially or completely insensitive to the insecticide [14, 18, 21-24, 26, 30].

Acetylocholinoesterase, the key enzyme responsible for the correct functioning of cholinergic synapses, is an example of enzyme which becomes insensitive to insecticides as a result of changes in amino acid sequence. [33]. This enzyme insensitive to organophosphorus and carbamate insecticides was identified in mosquitoes (genus *Anopheles* and *Culex*), flies (genus *Musca* and *Drosophila*) [22], beetle (*Tribolium castaneum*) [26], human lice (*Pediculu shumanus*) [25], peach aphid (*Myzus persicae*) [13] and western flower thrips (*Frankliniella occidentalis*) [18]. Acetylcholinesterase insensitivity to insecticides can be caused by mutations in the *ace-1* gene encoding this enzyme [22]. Very often there are the combinations of several mutations, which cause a significantly higher level of resistance and wider range of compounds to which acetylcholinesterase is not sensitive [33, 34].

Other examples of target site resistance are insensitivity to cyclodiene insecticides connected with mutations in the genes encoding γ-aminobutyric acid receptors [27], and resistance to DDT and pyrethroids caused by mutations in the genes encoding the voltage-gated sodium channels [35, 36]. In the second case, intensive use the insecticides leads to the development of resistance to both compounds, which is known as knockdown resistance (KDR). It occurs in flies [21, 22], aphids [13], moths [37], lice [25], beetles [26], and wasps [14].

The pests often possess more than one mechanism of defence against insecticide toxicity, which leads to the development of so-called cross-resistance [24]. An individual may be resistant to a compound even if the population has never had contact with this compound [18]. One of the first documented cases of cross-resistance was *F. occidentalis* populations from the area of Denmark, Switzerland, and Kenya. Resistance to carbamate methiocarb in this population have been observed. Although they had not been previously exposed to this compound, they possessed resistance to organophosphorus insecticides developed as a result of the strong selection by acephate and dichlorvo [38].

The use of insecticides can also be inefficient because of behavioral resistance. Some insecticides lead to a change in the behavior of some insect species, which allows them to avoid the adverse effects of insecticides. Changes in behavior may affect insect activity (time and length of feeding), feeding space, and place of oviposition, or promote the passage to the stage of diapause (in the case of larvae) [26]. An interesting example of resistance was observed in *T. castaneum*. When the insecticides were used, the females copulated with several male partners. However, only the semen from the last copulation was used to fertilize. This behavior, an example of sexual selection, increased the chance that the ovum was fertilized by sperm derived from a male that was resistant to the insecticides [39].

Socioeconomic Costs

Globally each year about 3 billion kilograms of pesticides are used, with annual purchase price of up to $40 billion. However, despite the use of pesticides, pest damage in the United States amounts to 37% of the harvest, 13% of which is destroyed only by insects. If we calculated only the costs associated with the purchase of pesticides each dollar invested in insecticide gave back $4 in the harvest. Nevertheless, insecticides do not always reduce crop loss. From 1945 to 2000 in the United States a 10-fold increase in the amount of pesticides used was associated with an increased loss of crops from 7% to 13% [40].

The costs associated with the use of insecticides include direct costs (funds for the purchase of plant protection products) and indirect costs related to:

(i) utilization of pesticides and their storage containers
(ii) protection of insecticide storage locations
(iii) funds disbursed to the control of food products and drinking water content to the insecticides
(iv) costs of treatment of poisoned people and animals
(v) social funds for helping people who are not able to fully function in society because of the diseases caused by insecticides.

In addition to the costs associated with the use of pesticides, environmental costs associated with the negative influence on the natural ecosystems have to be included [40-44].

Indirect costs are usually external to the person deciding on the use of insecticides, because these costs are divided into the whole society and not directly affecting the person using insecticides [45]. There are no tools to force the farmer to calculate all costs associated with the use of these compounds [44], and if indirect costs are not taken into account, the determining of benefits and losses of insecticide use can be falsified [43]. The use of insecticides is a typical example of the negative externalities where a significant part of the costs are shared by the whole society [44].

Insecticides are one of the main factors responsible for poisoning humans [46-50]. Every year there are from 250,000 to 370,000 plant protection compound poisonings globally. The acute poisonings are a particular problem in developing countries where these compounds are common and widely used in subsistence farms. In the case of developed countries, a bigger problem is chronic poisoning [46, 51].

A significant group of acute poisonings are suicidal. Organophosphorus insecticides account for 25% of suicides in the whole world [51], and in some countries it can be 50% (for example in Sri Lanka) [52]. It is the consequence of easy access to these chemicals and lack of control in trade of these compounds that can be bought without any restrictions [46, 51, 53].

A large part of insecticides is characterized by high persistence, which leads to wide distribution at various levels of ecosystems – air, water, soil, as well as living organisms. Most of them have lipophilic properties and in this way they are very easily accumulated in different tissues [54, 55].

Groups of insecticides that have the highest tendency to be accumulated in human tissues are organophosphorus and organochlorine compounds. The most vulnerable tissues to this process are adipose and nervous tissue. These compounds were detected also in relatively high concentrations in blood and milk. Over time, the balance between the amount of insecticide accumulated in the tissue and the amount taken into the body and excreted has been determined. In this way the measurement of insecticide content in tissue shows how the organisms are exposed to insecticides occurring in the environment [54].

Waliszewski et al. [54] checked the content of organochlorine insecticides in 60 breastfeeding women from Mexico's Veracruz region. They analyzed the adipose tissue, milk, and maternal and cord blood. Hexachlorobenzene (HCB), isomer β heksachlorocykloheksane (HCH), and isomer p,p-DDE was detected in more than 90% cases in milk and adipose tissue, and HCB and p,p-DDT in at least 98% of maternal and cord blood. In similar studies, where the cord blood from 1,196 children born between 2004 and 2006 in Belgium was tested, it was shown that in 99% and 70% of new-born cases the blood contained p,p-DDE and HCB, respectively [56]. Porta et al. [57] showed that the blood of 88% of 1,347 inhabitants of Catalonia (Spain) contained p,p-DDT despite prohibition of the use of this chemical in 1977. The study also showed that the level of these insecticides is significantly higher in women than in men, which is particularly risky [57]. The presence of DDT and its metabolites in maternal and cord blood has had a negative impact on the anthropometric parameters in infants. They revealed that high concentrations of insecticides and their metabolites in maternal blood correlated with a decrease in the size of head circumference, weight, height, and crown-heel length in neonatal and weight loss of placenta [58].

Human exposure to organochlorine insecticides also causes disorders in the hormonal system. These disorders, in particular, are related to sex hormones – oestrogen, androgen, and thyroid hormones. Many organochlorine compounds have oestrogen-like effects. These compounds disturb the balance in hypothalamus – pituitary – thyroid axis. Increasing the concentration of DDE in the maternal blood raised TSH levels in cord blood of male new-borns, which was shown by Freire et al. [59]. DDT and its metabolites affected the secretion of placenta hormones. Treatment of the placenta cells by DDT and its metabolites increased progesterone secretion and decreased the human chorionic gonadotropin [60]. The mechanism of action is probably related to the influence of DDT and its metabolites on aromatase activity – an enzyme that converts dehydroepiandrosterone to oestradiol. In this way the conversion of progesterone to oestrogen was blocked, which led to increased secretion of progesterone. As it was shown, DDT and its metabolites also have estrogenic and antiandrogenic activity [61].

Insecticides introduced into the body are transformed in various ways. Organochlorine compounds are metabolized by a_1 and b_2 cytochromes. That was associated with increased production of free radicals and reduction in glutathione levels [62]. Insecticides contributed to the generation of oxidative stress. For example a rise in non-enzymatic oxidative stress markers in pregnant women blood was connected with a high concentration of insecticides [63].

Chronic heptachlor and its epoxy metabolite poisonings raise the risk and progression of breast cancer, promote cryptorchidism in males, and raise the risk of developing Hodgkin's lymphoma [62]. There were also reports suggesting insecticide participation in Parkinson disease, especially compounds that act neurotoxically on the dopaminergic system [64]. In addition, organochlorine insecticides and pyrethroids are involved in the aetiology of multiple sclerosis [65]. Some insecticides have carcinogenic properties unrelated to genotoxicity (direct DNA damage) but related to deregulation of the immune system. For example, children's leukaemia occurred with higher frequency in children exposed to organochlorine insecticides and pyrethroids [66].

Negative Impact on Ecosystems

Effects of insecticides used in agriculture are not limited only to the area of fields. The great part of these compounds always penetrates into the surrounding ecosystems [5, 67, 68]. The influence on ecosystems is associated with:

(i) impacts on non-target organisms (causing disorders balance of the population)
(ii) pollution of surface waters [5] and groundwater
(iii) air + pollution
(iv) soil pollution [13].

Redistribution of insecticides can be a result of:

(i) adsorption to the surface of soil and plant particles
(ii) adsorption on soil particles and moving with eroded soil by air and water
(iii) dissolution in water and then uptake by plants and accumulation in their tissues
(iv) dissolution in water and exposure to surface runoff or leaching
(v) evaporating and escaping into the atmosphere [4].

It should be noted that in this process the physico-chemical properties of insecticides, such as solubility, polarity, and dissociation constant, as well as the weather conditions (temperature, rain, sunlight) and terrain are very important [69]. One of the main systems affected by insecticides are aquatic ecosystems. Numerous plant protection products have high mobility and with water are washed from fields to different types of water ecosystems, such as canals, streams, rivers, ponds, lakes, and finally seas and oceans [70]. Insecticides affect both freshwater and marine ecosystems and impact invertebrates, vertebrates, aquatic plants, and microorganisms, including bacteria, phytoplankton, and zooplankton [71-77].

Insecticide toxicity to aquatic organisms may be reflected in problems with ecosystem functioning by disturbances in the flow of energy and the carbon cycle [73], and lead to reduction of species biodiversity of aquatic ecosystems. A decline in the total number of species [74] as well as changes in the proportion of abundance between individual groups of animals can be observed. More sensitive species (mayflies (*Ephemeroptera*), caddisflies (*Trichoptera*)) are eliminated while the less sensitive (flies (*Diptera*), beetles (*Coleoptera*) and snails (*Gastropoda*)) to the insecticides become expansive species [70]. A good example of imbalance was an increase in the number of southern leopard frogs (*Rana sphenocephala*) in areas where carbaryl was applied. Although it did not act directly on the frog it effectively eliminated species that prey on frogs and in this way it reduced its mortality [78]. Effects on aquatic organisms can be related also to reducing fertility [74], abnormal developmental processes [77, 79], and behavioural changes [71, 80].

Another group which is particularly at risk for synthetic insecticides are birds [81, 82]. One of the most known effects is the influence of DDT on the weakening of the shell structure of birds' eggs, which makes brooding impossible [83]. Birds take insecticides during alimentation, for example with dead insects (after insecticides spraying), with sprayed seeds and ground and the birds of prey with the body of their prey because the insecticides are accumulated with high efficiency in animal tissue [81].

Victims of insecticides also include harmless insects, mainly due to the low selectivity of insecticides [73]. A good example is the honeybee (*Apies melifera*), which in the U.S. alone generates $15 billion [84]. Organophosphorus insecticides increase bee mortality on eggs and larvae, which leads to the disturbances in structure of the population, causing high financial losses [81].

An Alternative for Synthetic Insecticides

The biologically active peptides produced by insect neurosecretory cells are lately intensively studied in the context of use as bioinsecticides [9]. These compounds normally may act as peptide hormones, neurotransmitters, and neuromodulators, and they regulate the most crucial physiological (development, growth, reproduction, metabolism) as well as behavioural processes in insects [85, 86]. Important is that the same hormonal signal that can act differently, depending on the stage of insect life cycle, sex, and obviously, species [87]. Because of this, biologically active insect peptides seem to be a good alternative to synthetic insecticides. Understanding the mechanisms of insect neurohormonal regulation allows us to precisely design peptidomimetics, pseudopeptides, and small molecular weight compounds able to disrupt physiological processes regulated by native molecules. An increased amount of data available about the structural and functional properties of biologically active insect peptides and their receptors stimulates the further development of this alternative approach to conventional insecticides [88]. Insect peptides that can be potentially used as bioinsecticides are presented in Table 1.

However, there are limitations associated with the use of the native hormone molecule as bioinsecticides. Based on available data these compounds are modified for overcoming such problems as:

(i) instability of the peptides in the environment (sensitivity to temperature, pH),
(ii) hydrophobic insect cuticle impermeable to most compounds
(iii) rapid degradation of hormones in the insect digestive tract by peptidases present there [9].

In the next part of this article, information about several groups of insect hormones tested as potentially biopesticides will be presented. Peptides, such as trypsin modulating oostatic factor (TMOF), pheromone biosynthesis activating neuropeptides (PBANs), pyrokinins (PKs), sulfakinins (SKs), and allatostatin (ASTs), as well as their analogues, have been extensively studied to change the image of modern agriculture based on chemical pesticides.

Trypsin Modulating Oostatic Factor

TMOF is a decapeptide which blocks the biosynthesis of trypsin- and chymotrypsin-like enzymes in midgut epithelial cells in female and larvae of insects. [89]. Thus far, it was isolated from mosquito *A. aegypti* [90] and grey flesh fly *Neobellieria bullata* [89]. In adult females, TMOF blocks digestion of blood in the gastrointestinal duct, which results in inhibition of egg development in the ovary. In larvae, this factor blocks the digestion of protein nourishment, causing malnutrition and death. TMOF taken *per os* is easily absorbed by epithelium to the haemolymph and then acts on specific receptors located on the basal surface of epithelial cells and leads to arrest biosynthesis of trypsin and to termination of oocyte development [90].

The main problem in the application of TMOF as insecticide is low solubility in water. So there are attempts to use other vectors to introduce it into the insect body. One of the methods is the use of the tobacco mosaic virus as a TMOF gene carrier. After viral infection the plant cells began to produce TMOF, which got into the insect *per os* with plant nutrients. This way allowed reducing lethal doses for mosquito larvae from nanograms to picograms TMOF *per* larva [91]. TMOF analogues presenting in *Lepidoptera* and in flies from the *Sarcophagidae* family also inhibited the syn-

Table 1. Insect peptides potentially used as bioinsecticides.

Family of peptides	Abbreviation	Characteristic active sequences
Trypsin modulating oostatic factor	TMOF	YDPA
Pyrokinins/pheromone biosynthesis activating neuropeptides	PK/PBAN	PXPRLa
Sulfakinins	SK	X(SO_3H)GHMRFa
FGLa-allatostatins (A-type allatostatins)	FGLa-AST (A-type AST)	F/YXFGLa
Lepidopteran myoinhibitory peptides (B-type allatostatins)	MIP (B-type AST)	W(X6)Wa
PISCF-allatostatins (C-type allatostatins)	PISCF/AST (C-type AST)	PISCF
Kinins	K	FXXWGa

thesis of trypsin in intestinal cells after ingestion of blood or milk protein by the insect [92]. So far, several analogues were synthesized in order to determine the shortest core sequence of TMOF responsible for biological activity. Studies confirmed that the active fragment is tetrapeptide YDPA. Its biological activity has been similar to the activity of native TMOF [93]. Other chemical modifications used to increase TMOF efficiency and resistance to degradation by proteases was conjugation TMOF molecule with aliphatic polyethylene glycol (addition of methyl(ethylene glycol)7-O-propionyl to Lys in TMOF-K) [94]. In this way, it has obtained TMOF-K-PEG_7P, which was about 6-fold more toxic for *A. aegypti* mosquito larvae than TMOF. It probably was a result of significantly higher resistance to degradation by insect proteases [95]. Research on this peptide has shown that it is possible to use as an insecticide against mosquito larvae and probably other aquatic insect larvae [9].

Each compound used as an insecticide should minimally affect vertebrates, including humans. Previous studies indicated that TMOF can be degraded *in vitro* by leucinoamido peptidase (vertebrate pancreatic enzyme involved in proteins digestion). Toxicological tests on mice showed no adverse effects when TMOF was dosed *per os*. A similar result was obtained in the case of ducks and rabbits, which were transdermally treated by TMOF [96]. So it seems to be safe for humans or other mammals.

Pyrokinins

Pyrokinins are the group of insect neuropeptides which have common five amino acid sequence –FXPRL at the *C*-terminal end of the peptide chain. In position X it may be T, S, G, or V [97]. Since the first isolation of pyrokinin (leukopyrokinin) from brain extract of *Leucophaea maderae* in 1986, already several bioanalogues of this peptide in different species of insects have been detected and characterized [98]. Peptides belonging to this family are highly structurally variable and they are composed of few (PKs) to tens of amino acids like PBAN, diapause hormone, or melanization and reddish coloration hormones (MRCH) [99]. These neuropeptides are produced mainly by neurosecretory cells located in the suboesophageal, abdominal, and thoracic ganglions of ventral nerve cord and then secreted into the haemolymph [98]. Their physiological functions are regulating pheromone biosynthesis, stimulation of the intestinal muscles contraction, regulation of secretion by Malpighian tubules, controlling the moulting process, and releasing digestive enzymes in the intestine [9].

A wide range of processes controlled by pyrokinins, particularly by PBANs, entail that these hormones are the main candidates for natural compounds used in insect control [97]. PBANs are a group of 33 amino acid peptides that regulate pheromone biosynthesis pathways – substances responsible for attracting the opposite sex [100]. The main impediments in applying PBANs as potential bioinsecticides are their hydrophilic nature, making it difficult to penetrate through the hydrophobic cuticle and quick degradation of PBANs in the insect digestive tract by intestinal peptidases [101]. The alternative are synthetic analogues of these compounds, obtained by the addition of various chemical groups, which changed their physical and chemical properties [99]. Nachman et al. [101] developed a bicyclic PBAN analogue: cyclo [ASN^1] Leuma-PK and its *N*-terminal linear counterpart [2-8, Asn^9], Leuma-PK. The linear form showed no activity at concentrations lower than 100 nM, while the cyclic form showed a 70% level of activity compared to the parent natural PBAN at 100 nM concentration, but it was less susceptible for degradation by insect peptidases.

Another use of PBAN as an insecticide is synthesis of PBAN's antagonist. The studies of pyrokinins' receptor structure and identification of the domain responsible for interaction with the ligand present a possibility to construct molecules that effectively block the binding site of the peptide in the receptor and in this way the reactions initiated by peptide hormones are arrested. Such modifications were intended to produce PBAN antagonists by cyclization of molecules using the side chains, which led to conformational changes of the peptide [97]. A structure activity relationship study carried out by Altstein et al. [102] showed that the shortest peptide fragment responsible for the activity of the molecule is *C*-terminal sequence -FSPRLa. They substituted the serine or arginine by D-isomer of phenylalanine.

In this way, they received linear antagonist [D-Phe30]-PBAN-(28-33) [102]. This antagonist with D-Phe at position 30 was used to construct peptides of a backbone cyclization library. The Pro residue was replaced by the N-α-(ω-aminoalkyl)-Gly building unit, with various lengths of the alkyl chain, and the ω-amino group of the Gly building unit was connected to the *N*-terminal amino group by a dicarboxylic acid spacer of various lengths [103]. By this approach, compounds with up to 96% antagonistic activity were found; the most active peptide had an alkyl chain length of 2 and a spacer chain length of 3 carbons [102].

Sulfakinins

Sulfakinins (SKs) are multifunctional neuropeptides found in many insects. They were first isolated from head extracts of the cockroach *L. maderae*, exhibiting myostimulatory activity. SKs display high sequence similarity with the gastrin/cholecystokinin neuropeptides in mammals. After their discovery, SKs have been identified in various insects, such as locusts, crickets, and flies via either peptide isolation or gene prediction [104]. The observed immunoreactivity suggests that SKs function as central nervous system neurotransmitters in all insects investigated and as regulators of alimentary tissues in some insects, either through direct innervation or through their actions as hormones [105]. Currently, sulfakinins have been known mainly as the gut and heart contraction modulators in several insect species. They also inhibit food uptake in locusts and cockroaches and stimulate the release of the digestive enzymes in the scallop *Pecten maximus* and insect *Rhynchophorus ferrugineus* [106].

The most unique structural feature of the sulfakinins is the tyrosine sulphate residue Tyr(SO_3H), which is required for both the hindgut contractile and food intake-inhibition activity. However, the Tyr(SO_3H) group is susceptible to hydrolysis, particularly at acidic pH, and is relatively difficult to synthesize [107]. So the main step in design and synthesis of new SKs analogues, stable in the environment and that could potentially suppress the feeding behaviour of insect pests, is the identification of a readily available mimic of the unstable Tyr(SO_3H) residue. A good solution was synthesis of SKs analogues with replacement the Tyr(SO_3H) group by α-aminosuberic acid. Analogues with this modification have shown similar response to the natural sulfakinins and have been less sensitive to degradation [108]. Yu et al. [109] synthesized several analogues of SKs where α-aminosuberic or isosteric norleucine was incorporated into the polypeptide chain. The analogues containing α-aminosuberic acide reduced the food intake at similar levels as native SKs and the analogues with isosteric norleucine showed about 20% less activity.

Allatostatins

Juvenile hormones (JH) produced by *corpora allata* (CA) play the main role in growth, metamorphosis, and reproduction in most insect species [110]. Because of this any compound that influences JH production can be regarded as a potential agent in insect control [111]. Allatostatins (ASTs) can inhibit *in vitro* biosynthesis of JH by the CA and thereby control insect maturation and egg production. Peptides belonging to the ASTs family also exhibit other biological properties, such as modulation of myotropic activity in both gut and heart, inhibition of vitellogenin production in body fat, and stimulation of carbohydrate enzyme activity in midgut. These abilities to influence the number of physiological processes raise the possibility that ASTs could have potential new compounds use in insect pest control [112, 113].

Currently, there are three allatostatin families FGLa/ASTs, MIPs/ASTs, PISCF/ASTs (also called as A-, B-, and C-type allatostatins, respectively) that inhibit JH biosynthesis. The FGLa/ASTs allatostatins were originally identified from the cockroach *Diploptera punctata*, and are characterized by a common *C*-terminal pentapeptide (F/YXFGLamide), which is the minimum structural requirement for biological activity of the molecule [111, 114]. These peptides inhibit the biosynthesis of JH *in vitro* only in cockroaches and crickets. In all other species where FGLa allatostatins are found, including blowflies, bees and stick insects, they do not have any effect on the biosynthesis of JH. However, they affect visceral muscles by inhibiting its spontaneous activity, inhibiting the heartbeat of some cockroach species, and inhibiting vitellogenin synthesis *in vitro*. In other cockroaches, the activity of the two carbohydrases, amylase and invertase, was stimulated [9]. The FGLa/ASTs are related to vertebrate somatostatin, galanin, and opioid peptides [28].

The MIPs/ASTs were isolated from the cricket *Gryllus bimaculatus* and have been shown to be allatostatic in crickets [114]. However, the first 'cricket' allatostatin (previously B-type AST) was identified in *L. migratoria,* where it suppresses spontaneous contractions of hindgut and oviduct *in vitro* but is not allatostatic [9]. ASTs from this family are characterized by a similar structural motif with tryptophan residues at positions 1 and 8 from the *C*-terminus, and they sometimes are referred to as the W(X6)Wamide peptides family [115].

The third group of allatostanin, PIS/ASTs – (previously C-type) are *N*-terminally blocked peptides. Characteristic features of this group are *C*-terminal sequence PISCF-OH and intramolecular disulphide bridge between the *C* residues at positions 7 and 14. The first of these ASTs was identified in the tobacco hornworm *M. sexta* on the basis of its allatostatic activity [115, 116]. Moreover, genes encoding PIS/AST peptides have been identified in the moths *Pseudaletia unipuncta* [117] and *Spodoptera frugiperda* [118], as well as in *D. melanogaster* [119] and *T. castaneum* [120]. In case of *D. melanogaster*, allatostatin receptors are related structurally to the mammalian opioid/somatostatin receptors family [121].

The main problem in using the allatostatins as bioinsecticides is instability in the environment and degradation by insect proteases as well as poor penetration through the insect cuticle. There are designed different analogues that are not susceptibile to degradation. In the case of PIS/ASTs, the sequence responsible for biological activity is pen-

tapeptide F/YXaaFGLamide [9]. Kai et al. [112] synthesized AST(b)Φ2 analogue of Dippu-AST 1, in which Tyr^1 was replaced by 3-phenylpropanoic acid. This modification resulted in an analogue that showed resistance to catabolism and exerted high activity. Other analogues where the F/YX fragment was mimiced by aromatic acids showed similar or higher biological activity in inhibition of JH synthesis. All those analogues were insensitive to degradation. Another modification used to mimic Xaa in the F/YXFGLamide sequence was incorporation of succinic acid. Some analogues with incorporated aromatic or succinic acid showed also some effect *in vivo,* while basic pentapeptide had no effect. Similar results were obtained by Xie et al. [111]. They showed that the most important amino acids for the ability of the *C*-terminal pentapeptide to inhibit JH biosynthesis were Gly^4 and Leu^5. Tyr^1 and Xaa^2 could be replaced by an aromatic group and an appropriate length of linker.

Conclusions

Agriculture has a strong impact on the environment, especially due to use of synthetic plant protection agents with a wide spectrum of negative effects on ecosystems. Problems with pollution caused by these compounds are a challenge for intensive developing agriculture. It needs new solutions for plant protection that are efficient but above all more environmentally friendly. Good candidates for modern insecticides seem to be biologically active peptides. As was said before, these peptides regulate the most life processes in insects and have a lot of desirable features for modern insecticides, such as:

(i) high efficiency (working well in low concentrations)
(ii) no tendency to accumulate in the environment
(iii) low toxicity for vertebrates
(iv) not poisoning humans
(v) large diversity of acting mechanisms
(vi) high number and variety of isoforms in each group.

These features and intensive studies on understanding physiological regulation of the life processes in insects give us hope that in the future agriculture will develop environmentally and human friendly compounds for plant protection.

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