PROMISING COMPONENTS OF PHYTOBIOTICS FOR ANIMAL HUSBANDRY AND VETERINARY MEDICINE WITH DIRECT ANABOLIC AND ANTI-STRESS EFFECTS

Review article

Timofeev N.P.[1], *, Bindasova T.N.[2]
[1] ORCID : 0000-0003-4565-7260;
[1, 2] CF "BIO", Koryazhma, Russian Federation

* Corresponding author (sciens[at]leuzea.ru)

Abstract

The research goal was to summarize the results and trends in the use of biologically active substances synthesized by plants in the diet of farm animals to improve and stimulate their growth, identify limitations in industrial use, and offer promising components for producing phytobiotics with improved qualities. Promising new and unconventional plant species and their active substances for producing phytobiotics with improved qualities based on isoquinoline alkaloids, saponins, and ecdysteroids were analyzed. The research showed that ecdysterone-containing phytobiotics from the plants *Rhaponticum carthamoides* and *Serratula coronata* were alternative substances compared to prohibited synthetic androgenic and estrogenic hormonal stimulants.

Keywords: feed additives, phytobiotics, ecdysteroids, ecdysterone, anabolics, cyanotis, achyranthes, pfaffia, leuzea, serratula.

ПЕРСПЕКТИВНЫЕ КОМПОНЕНТЫ ФИТОБИОТИКОВ ДЛЯ ЗООТЕХНИИ И ВЕТЕРИНАРНОЙ МЕДИЦИНЫ С ПРЯМЫМ АНАБОЛИЧЕСКИМ И АНТИСТРЕССОВЫМ ДЕЙСТВИЕМ

Обзор

Тимофеев Н.П.[1], *, Биндасова Т.Н.[2]
[1] ORCID : 0000-0003-4565-7260;
[1, 2] КХ «БИО», Коряжма, Российская Федерация

* Корреспондирующий автор (sciens[at]leuzea.ru)

Аннотация

В обзоре обобщены достигнутые результаты и тенденции в использовании биологически активных веществ, синтезируемых растениями, в рационе сельскохозяйственных животных для оздоровления и стимулирования их роста, выявлены ограничения при применении и предложены перспективные компоненты для производства фитобиотиков с улучшенными качествами. Проанализированы перспективные новые и нетрадиционные виды растений и их действующие вещества для фитобиотиков на основе изохинолиновых алкалоидов, сапонинов и экдистероидов. Показано, что экдистерон содержащие фитобиотики из растений *Rhaponticum carthamoides* и *Serratula coronata* являются альтернативными субстанциями в сравнении с запрещенными синтетическими андрогенными и эстрогенными стимуляторами гормонального действия.

Ключевые слова: кормовые добавки, фитобиотики, экдистероиды, экдистерон, анаболики, цианотис, соломоцвет, сума, левзея, серпуха.

Introduction

The basis of the high productivity of animals is balanced normalized feeding. It satisfies the need of animals for nutrition elements (proteins, fats, carbohydrates, amino acids, vitamins, macro-, and microelements) against the background of their safe, sanitary, and hygienic maintenance. Simultaneously, the growth and productivity of animals are closely related to the functional activity of the nervous, immune, and endocrine systems. With industrialized livestock, stress and immunodeficiency precede many diseases and cause pathological conditions of varying severity, reducing the quantity and quality of livestock products [13, P. 1]. Additionally, contamination of the microflora of the stomach with pathogenic microorganisms and feed with synthetic and natural toxicants comprehensively affect the health of the animal. It affects its immunity and severely limits the actualization of genetic potential in practical animal husbandry.

To combat bacterial infections in animal husbandry, since the early 1950s, antibiotics, producers of fungi (e.g., penicillin), or bacteria (e.g., tetracycline) have been added to the feed. They can be synthetic or semi-synthetic substances [42]. However, as stated in the official document of the World Health Organization Regional Office for Europe, *Tackling antibiotic resistance from a food safety perspective in Europe*, as a result of the excessive and global use of antibiotics, bacteria in humans and animals have developed resistance to these drugs, as a result of which infections that normally respond well to antibiotic treatment become difficult and sometimes impossible to cure [44, P. 14].

The negative effects of antibiotics are greater deposition of subcutaneous fat instead of dietary protein (deterioration of meat quality); with constant use, it is necessary to increase doses, but which, on the contrary, reduces productivity. Another issue is that antibiotics in the body of animals tend to accumulate in individual organs and tissues. During the culinary processing of such products, some antibiotics are not destroyed. The third issue is that antibiotics can weaken the animal's body, concerning adverse environmental factors. Therefore, their use is not recommended for breeding animals. Antibiotics are potent substances that are released according to list B. It is forbidden to take them for other purposes and mix two or more types [42].

Since January 1, 2006, all types of feed antibiotics have been completely banned in the EU countries, except for the ones prescribed by veterinarians [11]. In the USA, antibiotics have been banned since January 1, 2017, as a result of the adoption of a new Veterinary Feed Directive of the FDA [7]. Similarly, restrictions on the use of antibiotics have also been introduced in Asian countries such as Korea, Vietnam, and China, as well as in Australia and Latin America [12]. In other countries, the use of feed antibiotics is not prohibited thus far. However, they will prohibit them in the near future by replacing them with herbal remedies.

Simultaneously, in the 1960s and 1980s, tranquilizers (psychotropic drugs with hypnotic and sedative effects) began to be used to protect against stress [42]. For the accelerated growth of muscle mass of animals in the UK, Canada, Australia, New Zealand, and several other European countries, hormonal agents based on synthetic analogs of female and male sex hormones (estrogens, progesterones, androgens), as well as thyroid (thyroxine) and hypoglycemic (insulin) hormonal agents are used [19], [30].

Currently, all synthetic means of stimulating the growth and productivity of animals (hormonal anabolic, tranquilizers, antibiotics) are banned in most countries or are under prohibition. Even their trace amounts can have a very harmful effect on human health [27]. It is also believed that many feed antioxidants of synthetic origin, used to level oxidative stress, can inhibit the immune system, which in turn leads to suppression of the mechanism of protection against pathogenic microflora, and therefore are prohibited in many countries.

Therefore, to significantly reduce the share of infectious diseases in animal husbandry, one should apply preventive measures more widely, switching to phytobiotics (alternative herbal antimicrobials) [11], [20]. Simultaneously, there is a demand for herbal substances that have a direct anabolic and anti-stress effect. They are economically advantageous for the manufacturer of products. Simultaneously, they are free from the disadvantages of chemically synthesized hormonal agents and tranquilizers without having issues with safety and toxicity.

Materials and methods

The research goal is the systematization of scientific literature data on natural biologically active substances in the composition of feed additives and their sources according to the criteria of anabolic and anti-stress effects and safety issues when used in animal science and veterinary medicine. The following tasks are set to achieve the goal:

1. Examining the assortment of plants and their active substances of phytobiotics in the global practice.
2. Analyzing the effectiveness and limitations (toxicity and safety) in the application of existing phytogenic feed additives.
3. Establishing promising bioactive plant components for phytobiotics.
4. Proposing new and non-traditional plants as industrial sources of ecdysterone from the introduced species of flora of the Russian Federation.

The search for literary sources (as of July 2021) was performed in the following scientific electronic libraries with built-in search engines: Wiley Online Library [45]; Pubmed [29]; Springer References [33]; eLibrary.RU [10]; TSNSHB [8]; EcdyBase [24]. The primary keywords for the search were feed additives, phytobiotics, anabolics, anti-stress substances, ecdysteroids, ecdysterone, ecdysten, 20-hydroxyecdysone, ecdysone, feed additives, phytobiotics, anabolics, anti-stress ecdysteroids, ecdysterone, ecdysten, 20-hydroxyecdysone, ecdysone, etc. The names of plants and their active substances were considered in Latin, Russian, and English. Guidelines on toxic and poisonous substances synthesized by plants in Europe, the USA, and Russia were studied.

Results and discussion

3.1. Phytobiotics: assortment of plants, active substances, and restrictions

Phytobiotics or phytogenic feed additives are products of plant origin used in animal feeding to improve health and stimulate growth and productivity. The primary mechanism of action promoting growth is the result of stabilization of feed hygiene and beneficial effects on the ecosystem of gastrointestinal microflora by controlling potential pathogens. Other mechanisms of action include increased appetite, digestibility, and absorption of nutrients, improved immune response, induction, or inhibition of metabolic enzymes. The antioxidant effect of some phytogenic compounds is crucial due to the protection of the quality of feed and products obtained from animals fed with these biologically active substances. The highest correlation of the decrease in the antioxidant defense system is observed in connection with the stress factors accompanying care and cultivation.

The plants used in practice usually belong to the following botanical families: Labiatae*;* Umbelliferae *(Umbelliferae, Apiaceae); composite flowers (*Asteraceae*, Compositae);* nightshade *(Solanaceae),* ginger *(Zingiberaceae);* cabbage *Brassicaceae).* The species composition of the crucial plants in the phytobiotics of Europe is as follows: anise *(Pimpinella anisum);* sweet basil *(Ocimum basilicum);* clove *(Syzygium aromaticum);* mustard *(Brassica nigra);* ginger *(Zingiber officinalis),* coriander *(Coriandrum sativum);* cinnamon *(Cinnamomum zeylanicum);* marjoram *(Origanum majorana);* mint peppercorn *(Mentha piperita);* fenugreek *(Trigonella foenum-graecum); Capsicum annuum; black* pepper *(Pepper nígrum);* parsley *(Petroselinum crispum); rosemary (*Rosmarinus officinalis*);* celery *(Apium graveolens);* cumin *(Thymus vulgaris);* thyme (Thymus vulgaris*);* horseradish *(Armoracia rusticana);* garlic *(Allium sativum),* etc. [20], [21], [26].

The most active secondary metabolites of phytobiotics belong to the class of terpenoids, flavonoids, glucosinolates, steroids, and saponins. Their effects are antimicrobial, anti-inflammatory, antioxidant, antiparasitic, and antiviral; they also include increased feed intake and digestibility of nutrients. Polycondensed phenols (tannins) bind proteins, polysaccharides, and other biopolymers and protect them from decomposition by the microflora of the stomach, have a tart astringent taste, and inhibit the growth of pathogenic microorganisms. Some polyphenolic compounds are metabolized in the gastrointestinal tract through bacterial enzymes, which play an essential role in the bioavailability of phenolic glycosides.

The antioxidant potential of medicinal, spicy, and essential oil plants is primarily associated with the concentration of flavonoids (quercetin, rutin, etc.), hydrolyzable tannins, proanthocyanidins, phenolic acids (benzoic, cinnamon, coumarin derivatives), phenolic terpenes (volatile essential oils), vitamins (A, C, and E), and carotenoids [41]. One of the disadvantages of the existing phytobiotics is that they do not have a direct anabolic effect and do not work under severe stress. With a combination of adverse factors, it is impossible to overcome the negative effect. The effect of the average daily weight gain is not constant, and the opposite (negative) effect is often observed. Negative indicators are usually manifested in conditions of highly stressful situations [12]. The components of existing phytobiotics are ineffective or weakly effective under temperature stress (24-34 °C versus low-temperature conditions of 6–8 °C in control) [14].

According to the international analytical data [12], [46], the increase in the average daily increase in poultry farming from the use of phytobiotics is usually +1%...+3%. In some cases, a zero result is obtained or a decrease by -2%...-3%. Similar efficiency results are obtained in pig breeding. Out of 26 experiments, positive results have been obtained in half of the cases (+1%...+5%). In other cases, negative gains are recorded (-1%...-7%). The feed dosages range from 0.1 g/kg (0.01%) to 2-5 g/kg (0.2%–0.5%). In pigs, productivity improvement is expressed on average by 2% in terms of average daily growth, by 3% in terms of feed conversion efficiency, and in the range from -5% to +9% in terms of body weight change [12].

Other limitations are the variability and inconstancy of the composition of phytobiotics. The composition of phytobiotics varies widely depending on the botanical origin, substance composition, and technological processing. Therefore, they are difficult to quantify. However, there is still no standardization of phytobiotics for active substances and attempts to perform this reveal cytotoxicity in very small dosages [25].

3.2. New and promising bioactive components in phytobiotics

Recently, due to the weak effectiveness of phytobiotics in stressful situations, plants containing potent substances have begun to be involved in research. Among them is *Macleya cordata*, which synthesizes isoquinoline alkaloids. The research results are carried out on sows (farrowing stress) and piglets (weaning stress) and demonstrate that in low doses, they can regulate the stress response [1].

However, isoquinoline alkaloids and plants containing them (any parts) are classified by regulatory authorities as toxic substances of natural origin by the European Food Safety Agency, as they can pose a danger to human and animal health [28].

The following plants with saponins and alkaloids have been used to modulate immune reactions as part of phytobiotics, and when one overdoses, there may also be health problems. These plants include alfalfa *(Medicago sativa*), *Echinacea purpurea*, *yucca* (*Yucca spp.),* Spanish licorice *(Glycyrrhiza glabra),* and *Ginkgo biloba* [12].

Saponins are substances of glycosidic nature. They dissolve well in water and cause hemolytic poisoning when they penetrate the blood. The five-volume U.S. Review of Toxic Plants indicates that echinacea and alfalfa can lead to kidney failure and renal acidosis. Echinacea can also cause allergic reactions, rash, or exacerbate asthma [3], [4]. Licorice contains glycyrrhizic acid, triterpene saponins, and hydroxycoumarins, and can cause life-threatening cardiac arrhythmias due to hypokalemia (like aloe and buckthorn berries). It also causes liver cirrhosis, pulmonary edema, and electrolyte and renal abnormalities [6]. Ginkgo biloba *(G. biloba)* has carcinogenic activity. Its leaf extract can cause liver cancer, thyroid adenoma, and leukemia [5].

Unfortunately, the set of cultures as phytobiotics is extremely limited in Russia. However, there is a rich gene pool of wild flora. Specific types of plants used in Russia as phytogenics are coniferous flour (fir, spruce, pine), Jerusalem artichoke, beetroot, carrot, pumpkin, alfalfa, and sea buckthorn [2]. One should note that most of these species were used as feed additives in Soviet times. They were vitamin and herbal flour from alfalfa, coniferous flour, Jerusalem artichoke, beets, carrots, pulp and cake from fruit and berry, and spicy-aromatic and essential oil plants [42].

Therefore, searching for new and unconventional plant species with unique properties is urgent. The goal is to find sources of new, highly effective natural compounds of preventive, therapeutic, anabolic, anti-stress, and adaptogenic orientation, which are simultaneously economically profitable. In other words, feed production from the viewpoint of animal science and veterinary medicine needs a significant expansion of the range of species. Simultaneously, attracted species should be combined with traditional cultures.

One of the promising directions for improving the feed production system is the inclusion of ecdysteroid synthesizing plants in it. In Russia, there are unique plant sources containing phytoecdysteroids (PES) as biologically active components (Fig. 1). Distinctive positive properties of phytoecdysteroids are not available in currently widely used phytobiotics. Feed additives with them relieve severe stress, which conventional phytobiotics cannot do, have a direct anabolic effect of influence due to interaction with estrogen receptors, and have pleiotropic (multiple) effects of action due to the influence on important genes. Their use in animal husbandry does not cause concern, as they are safe substances [9], [18], [34], [47].The most important among the representatives of the ecdysteroid class, based on the practical significance, availability, and biological activity, is the bioactive substance ecdysterone (20-hydroxyecdysone, ecdysterone, 20E) [9].

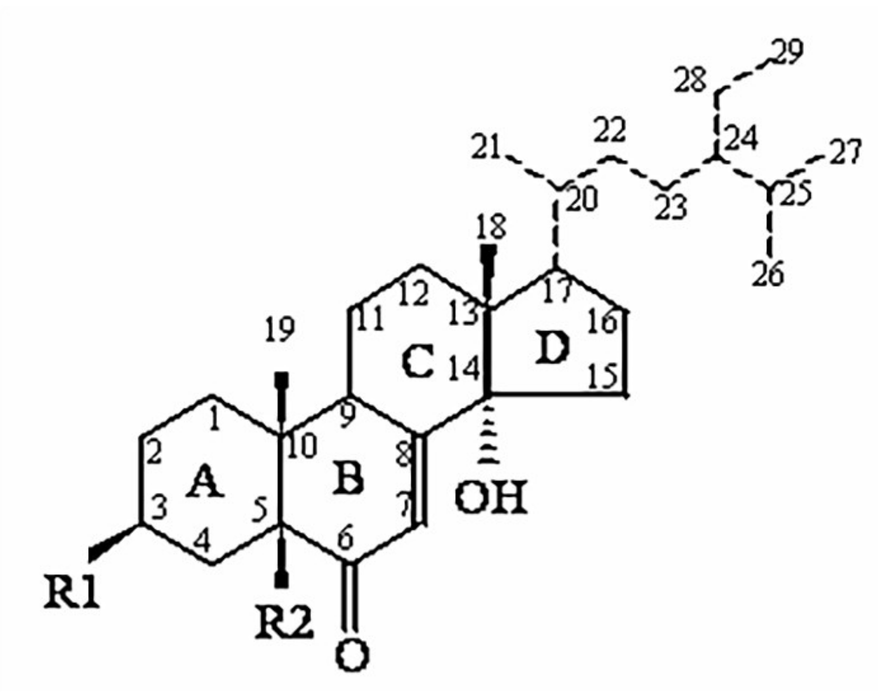

Figure 1 - General structural composition of phytoecdysteroids

Note: source [22]

3.3. Industrial sources of ecdysterone for phytobiotics

The sources of PES for phytobiotics are higher plants. Primary chemical synthesis of ecdysteroids is impossible in practice; biotechnological methods are ineffective. After several reproduction cycles, strains lose their ability to synthesize [9], [37]. In plants, after biosynthesis in the roots or leaves, PES are water-soluble and redistributed with the flow of assimilates through the phloem from adult organs to young and developing organs and tissues (apical parts, buds, and seeds) [22].

Despite the theoretical possibility of obtaining ecdysterone-containing substances from a variety of plants of the world flora, in fact, there are severe restrictions on attracting them to feed production for phytobiotics. It follows from an analytical review of the achievements and issues in the cultivation of ecdysteroid-synthesizing plants. As wild-growing raw materials for the production of ecdysteroids in various countries, rhizomes of ferns from the forests of Europe and South America (*Polypodium vulgare*, *P. lepidopters*) can be used; roots of plants of the amaranth family from the tropical forests of Brazil and the Amazon basin (*Pfaffla paniculata, P. glomerata*); needles of podocarps and yews from the highlands of China and Japan (*Podocarpus nakaii*, *P. macrophyllus*, *P. reichei; Taxus canadasis*, *T. chinensis*, *T. cuspidata*), seeds of endemic species of the genus *Ipomoea* growing on the southern slopes of the Himalayan mountains; aboveground biomass of perennial plants of the Commeline family located in China, India, and Taiwan on waterlogged mountain soils (*Cyanotis arachnoidea, C. vaga*). On the territory of Russia, the species potential of ecdysteroid-containing plants is mainly represented by the following species: varieties *Silene* (campion) and *Lychnis* (fireballs); *Coronaria flos-cuculi* (cuckooflower); *Helleborus purpurascens* (reddish hellebore) and *N. caucasicus* (Caucasian hellebore); *Paris guadrifolia* (herb Paris); *Ajuga reptans* (carpenter’s herb); *Sagina procumbens* (procumbent pearlsides); *Potamogeton* natans (tench weed) and *P. perfoliatus* (clasping-leaved pondweed); *Pulmonaria offlcinalis* (medicinal lungwort); *Butomus umbellatus* (flowering rush); *Androsace filiforms* (rock jasmine), etc. Generally, these plants are challenging to access, occur scattered or singly, only in wild form, and are not known in culture; their introduction has not been performed or is seriously hindered [39, P. 4].

Plants that, are considered in European countries as good sources of PES and deserve attention for the large-scale production of substances with ecdysterone in sufficient quantities and at a reasonable price are

1) species from the genera *Achyranthes* (from the *Amaranthaceae* family);
2) *Cyanotis* (*Commelinaceae* family);
3) *Pfaffia* (*Amaranthaceae* family);
4) *Leuzea/Stemmacantha/Rhaponticum* (*Asteraceae* family);
5) *Serratula* (*Asteraceae* family) [9].

According to experts and specialists [23], among the five mentioned groups, the plant that is primarily suitable for producing ecdysterone-containing substances is *Rhaponticum carthamoides* (aboveground and underground parts of the maral root*)*, and secondarily is cyanotis from China and other Asian countries *(Cyanotis arachnoidea and Cyanotis vaga).*

However, the value of plants is assessed not only by the ability to increase the synthesis of target substances but also by a low predisposition to the concentration of various toxic compounds of natural or anthropogenic origin. In this regard, representatives of the first three genera with a high content of ecdysteroids, including related species (*Cyanotis, Achyranthes*, and *Pfaffia)*, are classified as toxic agents and cannot be sold as part of feed additives, since they contain carcinogenic and kidney-damaging plant substances [15].

Studies with partially purified ecdysterone substances from *Pfaffia glomerata* have displayed their genotoxicity and cytotoxicity. It occurs due to the presence of other compounds (not PES) present in alcohol extracts of plants and not eliminated in the preparation creation [23].

The most acceptable in the conditions of Russia is the use of two plants in the composition of phytobiotics – carthamoides rhapontic (maral root) *Rhaponticum carthamoides* (Willd.) Iljin, 1933 (Fig. 2) and saw-wort crowned *Serratula coronata* L., 1753 (Fig. 3). In Russia, during the 60-year fundamental study of a collection of more than three thousand plant species by scientists from the Institute of Biology of the Komi Scientific Center of the Russian Academy of Sciences (Syktyvkar), these two species have been proposed for the industrial cultivation [31].

Figure 2 - Industrial source of ecdysterone from Rhaponticum carthamoides agropopulations

Note: age 16 years, h=1.5–1.7 m; photo of the author on the field of CF BIO

Figure 3 - The Serratula coronata agropopulation is a source of phytogenic substances with ecdysterone

Note: age 12 years, h=1.6–1.9 m; photo of the author on the field of KKh BIO

In the course of experiments conducted involving various animals at the N. V. Rudnitsky Federal Agrarian Scientific Center of the North-East (Kirov) in agro-industrial livestock complexes, the results are obtained indicating the absence of toxicity and any contraindications concerning water-alcohol extracts of these plants, as well as the presence of significant anabolic and protective activity under prolonged stress [16], [36].

These two tall-herbed perennial species have undergone a long stage of introduction. They synthesize large amounts of ecdysterone in leaf organs in *R. carthamoides* (0.3%-1.5% versus 0.03%-0.15% in roots with rhizomes) and apical parts of stems in *S. coronata* (0.7%-2.3%) [38], [39]. The biochemical composition and feed advantages and the possibility of their long-term cultivation in conditions of agrocenosis (up to 16 years and over) are shown and, for 2021, recognized internationally as the most important sources of phytogenically occurring anabolic and anti-stress substances [9], [23].

Ecdysterone, as part of the PES complex in their aboveground phytomass, stimulates protein synthesis in muscles and other organs in animals while preventing fat deposition. Unlike chemically synthesized hormonal agents, herbal stimulators of protein synthesis based on ecdysterone do not cause life-threatening side effects [9], [27], [32], [35]. Substances with ecdysterone from *R. carthamoides* are characterized by a high anabolic effect (10% to 40%). PES substances from *S. coronata* plants are characterized primarily by an anti-stress effect (up to 32%–35%). The anabolic effect is manifested to a relatively lesser extent (up to 5%-12%) [18], [37], [43], [47].

As a candidate for the PES plants described above, a new species of carthamoid rhapontic *Rhaponticum scariosum* Lam., 1753 — introduced to the European North of Russia from the subalpine meadows of Western Europe (Alps) can be attributed. During the reconnaissance study of ecdysterone accumulation by high-performance liquid chromatography (HPLC), it is found that the processes of biosynthesis in leaf organs increase with years of life to very significant values: from 0.19% (1st year) to 0.82% (10th year), interrupted only by steady frosts. Simultaneously, the increased level of ecdysterone biosynthesis is characterized by the absence of the undesirable mildly active ecdysteroid ecdysone (0.01%). Therefore, the extract does not require a purification from its impurity [40].

Conclusion

The review summarizes the achieved results and trends in the use of biologically active substances synthesized by plants in the diet of farm animals for health improvement and growth stimulation. The specific composition, active substances, effectiveness, and limitations in industrial use have been studied and personalized. Promising new and unconventional plant species and their promising components for producing phytobiotics with improved qualities based on isoquinoline alkaloids, saponins, and ecdysteroids are analyzed.

It has been shown that ecdysteroid-containing phytobiotics, where the primary bioactive component is ecdysterone (20-hydroxyecdysone), are alternative substances in comparison with prohibited synthetic androgenic and estrogenic hormonal stimulants. Simultaneously, they have a direct anabolic and anti-stress effect, are economically beneficial for the manufacturer of products, are free from the disadvantages of chemically synthesized hormonal agents and tranquilizers, and have no issues with safety and toxicity. It is possible to combine such substances with other antimicrobial agents in order to improve the bioavailability and prolong the action of the active substance ecdysterone.

Groups of plants considered good industrial sources of PES and deserve attention concerning the large-scale production of substances with ecdysterone are species from the genera *Achyranthes, Cyanotis, Pfaffia, Leuzea/Stemmacantha/Rhaponticum, Serratula*. A plant that is primarily suitable for obtaining an ecdysterone-containing substance is *Rhaponticum carthamoides.* It features a high anabolic effect. A significant anti-stress effect is crucial for PES substances from Serratula coronata plants. Representatives of the other three genera with a high content of ecdysteroids (particularly, *Achyranthes aspera, Cyathula capitata, Cyanotis arachnoidea, Cyanotis vaga, Pfaffia paniculata, Pfaffia glomerata*) are not officially allowed for sale as part of food and feed additives due to the content of prohibited substances, toxicity, and genotoxicity.

In the perspective of future research, plant species characterized by an increased content of ecdysterone and simultaneously a high yield of aboveground mass, do not synthesize toxic impurities, and are capable of long-term growth in agropopulations are of interest for the production of phytobiotics. Other important upcoming studies concern the examination of the variability and stability of the production of PES of selected plants during the life cycle in agropopulations. They also concern establishing the causes of the presence or absence of low- and low-active ecdysteroids in phytomass, the possibility of influencing the content of ecdysterone through the optimization of cultivation technology, and the experimental animal tests to establish a possible synergistic or antagonistic effect of ecdysterone containing substances with other components of phytobiotics, etc.

Конфликт интересов

Не указан.

Рецензия

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

Conflict of Interest

None declared.

Review

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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