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Extraction Method for Phytoncides from Plant Material.

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Abstract: This article discusses biologically active substances from plants—phytoncides—and their significance for humans. It presents methods for the extraction of certain subclasses of phytoncides, specifically essential oils, saponins, and glycosides. The scientific interest in these substances is driven by the relevance of finding alternative ways to combat airborne pathogens, protozoa, and insect pests that are safe for the environment.

Keywords: phytoncides, fractions, essential oils, saponins, glycosides, plant material.

Phytoncides (from the Greek φυτόν — "plant" and the Latin caedo — "I kill") are biologically active substances released by plants that can destroy or inhibit the growth and development of various pathogens, including bacteria, microscopic fungi, and protozoa. Phytoncides are part of the natural immunity of plants. The term "phytoncides" was proposed by B. P. Tokin in 1934 to refer to a class of volatile substances produced by plants that possess antimicrobial properties.

Phytoncides have various chemical natures; they essentially represent a complex of compounds—glycosides, terpenoids, tannins, essential oils, saponins, and other so-called secondary metabolites.

Phytoncides are of particular interest to modern science due to their antiseptic properties, and in some cases, their insecticidal activity, serving as an alternative to synthetic agents with the same spectrum of action.

The extraction of phytoncides from plant materials is a labor-intensive process. This is primarily due to the diverse chemical nature of these substances. The extraction method will depend on the chemical composition of the specific substance being extracted. Let's consider the main methods.

Extraction Method for Essential Oils. The most common method for extracting essential oils from plant materials is distillation. This method involves using water or steam to act on the plant material. There are two types of distillation: direct distillation, where the plant material is directly submerged in boiling water, and steam distillation, where the material is placed on a grid above boiling water, allowing steam to pass through the material. In both methods, the plant glands rupture, releasing the contained essence as vapor. This vapor, along with the steam involved in the distillation process, is collected in a tube that passes through cooling systems, after which it returns to a liquid form and flows into a separator. The vapor turns into an aqueous distillate, while the plant essence becomes essential oil.

Coniferous plants, citrus fruits, spicy plants, and richly flowering plants are the richest in essential oils.

Extraction Method for Saponins. Saponins are extracted from plant materials as follows: the ground dried raw material is treated with petroleum ether to break down the complexes of saponins with sterols. The resulting material is then processed with 96% ethyl alcohol to obtain a total saponin extract. Saponins are precipitated from the alcoholic solution using acetone. The obtained saponin fractions are purified through repeated precipitation to remove all impurities. Next, 70% ethyl alcohol is added, and centrifugation is performed, resulting in a diethyl-dried powder for further research.

Sources of saponins can include plants such as horse chestnut, field horsetail, St. John's wort, bird's knotweed, chicory, marshmallow, and others.

Extraction Method for Glycosides. Dried, ground plant material is extracted with 96% ethyl alcohol. The alcoholic extract is evaporated in a vacuum to a thick residue, which is then dissolved in 35% ethyl alcohol and filtered through aluminum oxide (with a ratio of thick extract to adsorbent of 1:5). The adsorbent is washed with 35% ethyl alcohol. Glycosides are extracted three times from the alcohol-water solution using chloroform, and then three times with a mixture of chloroform and ethyl alcohol (2:1). The combined alcohol-chloroform extracts are washed twice with distilled water and evaporated to dryness in a vacuum, yielding a purified total of glycosides.

Glycosides are abundant in the seeds of fruit plants, as well as in plants such as foxglove, belladonna, and nightshade, among others.

Both separately isolated fractions of phytoncides and their overall combinations are of great interest to science, as it is in the format of a series of complementary biologically active substances that phytoncides provide the natural immunity of plants.

Insect pheromones, discovered more than fifty years ago, can now become a safe and harmless substitute for pesticides and other harmful chemicals that are currently used in the fight against harmful insects that cause huge damage to agriculture.

The question arises, what is the essence of pheromones? How do they act on insect pests? How external environmental factors affect them, and most importantly, what are the prospects for the use of pheromones in agriculture.

Pheromones are biologically active substances, products of external secretion, secreted into the environment by insects, fish, animals, communication between organisms of the same species are some kind of volatile chemosignals capable of controlling neuroendocrine behavioral reactions, developmental processes, social behavior and reproduction. Pheromones alter the behavior, physiological and emotional state, and even the metabolism of different individuals of the same species. These substances, are means of regulation, play an important role in the communication of many species of insects, for example, ensuring the rapprochement of males and females during the breeding season, the concentration of insects on forage plants and in wintering places, or controlling the behavior and physiological processes of working individuals of social insects. Pheromones are found in animals of various taxonomic groups, from invertebrates to mammals. At present, insect pheromones are considered the most studied.

There are two main types of pheromones that differ in their effects: releasers and primers. The first type - which transmit danger signals between individuals of the same species. Usually releasers are human. Pheromones, providing chemical releasers, are able to induce an individual to take immediate action, for example, pheromones, highly volatile substances that spread through the air. The second type of the formation of special behavior and influence on other individuals, an example is the pheromones secreted by the queen bee, in order to suppress the sexual development of female bees, turning them into ordinary working bees. Primers are most commonly distributed by contact. At this time, releasers have been studied better than primers, by their example, several subtypes of pheromones can be distinguished, such as: attractants - these include sex pheromones and aggregation pheromones that stimulate the accumulation of insects; repellents - repelling pheromones; primers designed to stimulate pheromones that induce activity, for example, anxiety pheromones; deterrents that inhibit the reaction, etc. [5]

The source of pheromone in insects can be individual secretory cells scattered throughout the body or groups of them, which form a special organ of the pheromone gland. The ducts of the pheromone glands open on the surface of the body or in cavities that communicate with the external environment. Insects secrete pheromone in trace amounts: for example, a female codling moth (Cudiapomonella) releases only 9 nanograms of pheromone per hour. However, even this amount is enough for a male moth to smell and find a female in the crown of a tree. Insects perceive the smell of pheromones with the help of chemoreceptor sensilla - special receptors in the form of hairs, bristles or tubercles located on the antennae; their number on one antenna can reach 15 thousand. A very small amount of pheromone in the air is enough for an insect to respond. [4]

Usually, pheromones are not one substance, but a mixture of the main, predominant by weight component with small additives (minor components): they can contain more than 10 components. One substance can have several different functions. Pheromone molecules are highly volatile, quickly decompose under the action of atmospheric oxygen, moisture and light. In terms of chemical composition, insect pheromones belong to various classes of organic compounds, such as alcohols, ethers, terpenoids, steroids, aldehydes, heterocyclic compounds, and others.

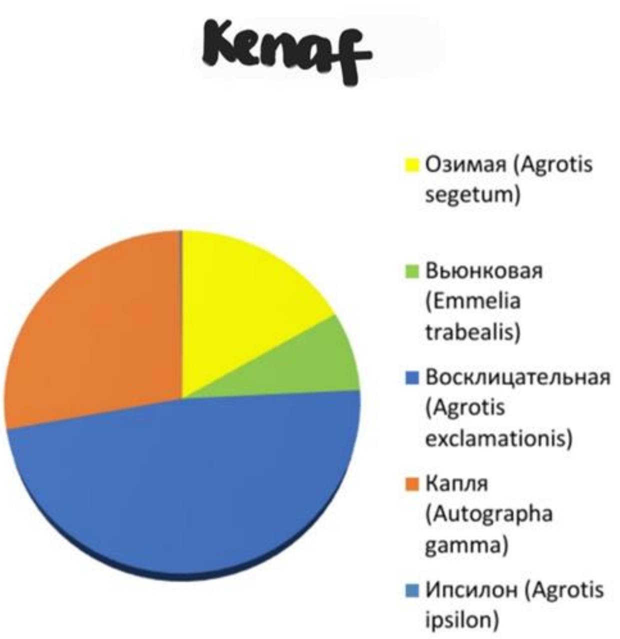
Knowing the chemical composition of the insect pheromone, it is possible to synthesize it in laboratory conditions. It is these synthetic analogs of sex and aggregation pheromones that can be used to protect plants from pests. The advantage of synthetic pheromones, which are used in micro doses, is their high species specificity and attractiveness. They are completely harmless to humans and the environment, and also act directly on the target species of insect pest.

There are two main areas of application of synthetic pheromones against harmful insects: First, monitoring. This suggests that the use of pheromones provides an opportunity to record such processes as the flight of pests, to obtain data on their numbers, or even the ability to determine the area of quarantine pests. Second, it provides an opportunity to prevent males from finding females, attract insects, and catch or destroy pest control. By saturating the air with synthetic pheromone, before they can find a natural source of pheromone. In both cases, the reproduction of pests is blocked.

However, in addition to the effect of pheromones on pests, it is necessary to consider the influence of environmental factors on the pheromones themselves. Taking into account the huge species diversity of insect pests and the complexity of the composition of pheromones, an urgent task is to develop universal methods for studying pheromone communication, which will save material, labor and time resources.

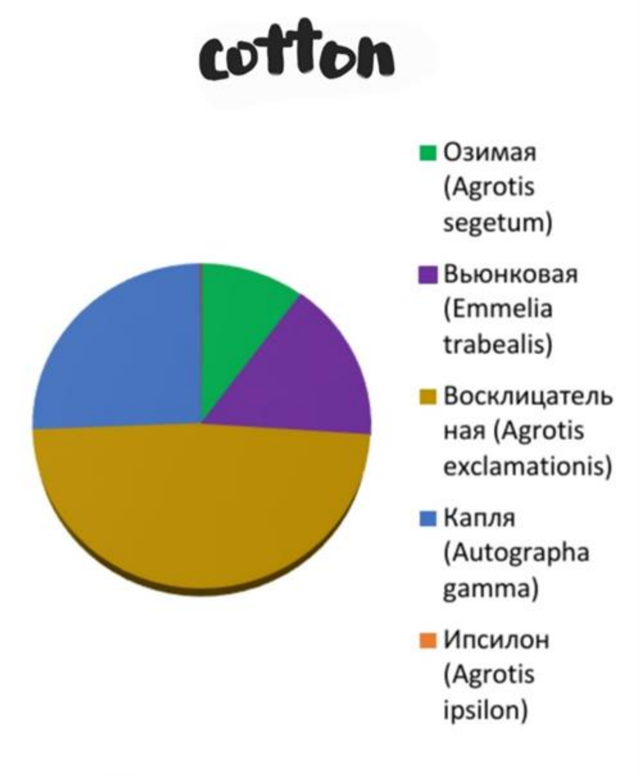
Dispensers with pheromones were placed in triangular traps made of laminated paper, which were placed in the fields at the rate of 1 trap per hectare at a height of 25 cm above the plants. The pipettes were renewed every 10 days. Observations were carried out over three years in the fields of cotton, kenaf, corn, alfalfa, red pepper, tomato and pumpkin. Based on the number of males of each species caught in pheromone traps, we calculated the indicators of the relative abundance of the species [4].

The number of the moth complex in agrocenoses of the cotton crop rotation



On the surveyed fields of cotton crop rotation (cotton, kenaf, corn, alfalfa), the complex of scoops, determined by the presence of pheromones, as differences were observed in individual years, as a rule, it is of the same type. which concerned mainly small species. Thus, in all areas the dominant species was the bindweed (Emmeliatrabealis), the subdominant exclamation moth (Agrotisexclamationis) (Agrotissegetut). There were no caradrina moths

(Spodopteraexigua) and corn and winter moths (Spodopterafrugiperda) in the corn and cotton fields. In a cotton field, bindweed (Emteliatrabealis), winter moth (Agrotissegetum), (Agrotisexclamationis), cotton moth (Helicoverpaarmigera), meadow moth (Mythimnaunipuncta), as well as gamma moth (Autographagatta), c. black (Xestia c-nigrum), ipsilon owl (Agrotisipsilon). The species diversity of moths in the cornfield was slightly less exclamatory: there was no cotton moth (Helicoverpaarmigera) and no upsilon moth (Agrotisipsilon).



In the alfalfa field, all types of scoops were identified, the pheromones of which were used. All kinds of scoops, whose pheromones were used during observations, (Spodopteraexigua) and (Spodopterafrugiperda) were also found in the fields of vegetable crops. In the Tashkent region, the number of scoops was in general, with the exception of caradrins of leafy corn scoops. On vegetable crops it was higher than in cotton crop rotation fields in Yangiyul region. In vegetable crops, as well as in agrocenoses of the Cotton crop rotation, the dominant species was the bindweed (Emmeliatrabealis), the subdominant (Agrotisexclamationis) was the exclamation winter (Agrotissegetum). So, one day after the installation of pheromone traps, 14.7 individuals were caught on red peppers, and 11 individuals were caught for tomatoes and alfalfa. At the same time, in the fields of alfalfa, tomatoes and red pepper, it was found on traps with winter pheromones of 6, 7.7 and 10.7 individuals, respectively. According to the available data, the generalized economic threshold of harmfulness is considered to be capture, on average, per one trap per day (night) (Agrotissegetum), which corresponds to a caterpillar density of 2.6 - 4.0 individuals per 1 m^2.

RESEARCH METHODS

Theoretical modeling and calculation of the physical characteristics of pheromone molecules were carried out within the framework of the density functional theory using the B3LYP functional, using the 6-31G ** and cc-pVDZ basic packages, implemented in the GAMESS-US program. The absorption spectra were calculated and the molecules were optimized in the excited state by the

TimeDeredent method. The assessment of the effect of thermal action on pheromone molecules was carried out using a method based on the calculation of the modes of normal vibrations.

Figure 1 - Atomic structure of pheromones of lepidoptera insect

The geometry of the pheromones of lepidoptera insects allows the formation of conformers due to the rotation of the parts of the molecule relative to each other around the σ-bonds; therefore, at the first stage of the study, modeling and calculation of the properties of pheromones in various conformations other than linear structure were carried out. For all conformers, the energy and structural characteristics were determined in the ground and excited states, and the absorption spectra of electromagnetic radiation were calculated. Analysis of the data obtained showed that a change in conformation leads to a change in the total energy of molecules by no more than 24 kJ / mol, which is less than 0.001% of the value of the total energy of molecules. On average, the difference between the total energy of the conformers is 6 kJ / mol. An insignificant difference in energy between conformers indicates that pheromonesThe linear and twisted conformations corresponding to the absorption maximum with the corresponding values of the linear dimensions of the pheromone molecule, its electric dipole moment and length, correspond to Lepidoptera insects, the formation of certain conformations is not characteristic. maximum absorption. The influence of the geometry of molecules on the value of the electric dipole moment and the absorption spectra of pheromones was considered. [5] For oxygen-containing pheromones, a change in conformation leads to a change in the dipole moment by an average of 30% relative to the linear structure; for hydrocarbons, an increase in the dipole moment when passing from a linear to a maximally twisted conformation is up to 50%.

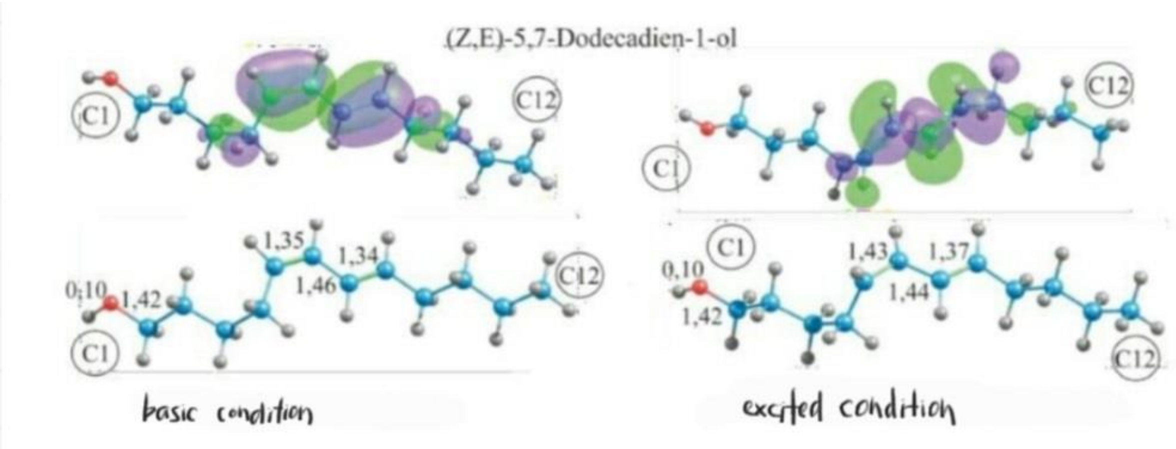


Figure 3 - Atomic and electronic structure of pheromones in the ground and excited state: carbon atoms are marked in blue, hydrogen atoms - in gray, oxygen atoms - in red.

Exposure to electromagnetic radiation leads to a change in the electronic and atomic structure of pheromones. For disparlyur, a pheromone of the gypsy moth (Lymantria dispar), which does not have multiple bonds in the structure, the change in the electron density upon absorption of radiation occurs in the region of the epoxy ring for all conformers. Calculation of the geometry of the disparlyur in an excited state shows similar structural changes for all conformations: the bond angles change in the epoxy ring, one of the C-O bonds increases on average to 0.9 A, which can further lead to its rupture and, as a consequence, to the opening of the cycle and the destruction of the pheromone. Most pheromones of lepidoptera insects belong to unsaturated compounds containing up to three double bonds in their structure. An analysis of the electronic structure of pheromone molecules shows that, regardless of the presence and type of oxygen-containing functional group, the redistribution of the electron density upon absorption of light occurs in the region of the location of double bonds and corresponds to the transition of an electron from n-bonding to n * - bonding orbitals (Figure 3). The bond lengths are indicated in angstroms (A).

Figure 3 shows the values of the lengths of chemical bonds in molecules of unsaturated alcohol and unsaturated epoxide in the ground and in the excited state.

The change in bond lengths occurs only in the area of multiple bonds and does not affect oxygen-containing functional groups. Similar structural changes occur in all unsaturated pheromones of Lepidoptera. An increase in the lengths of double bonds occurs on average by 0.1 A. Such a change in the bond length is unlikely to lead to the destruction of the initial atomic structure of molecules only under the influence of electromagnetic radiation, but will increase their reactivity and promote chemical reactions when interacting with air components. The interaction of the pheromone with the protein of the insect's olfactory receptor occurs according to the "key-lock" principle, that is, in addition to the chemical composition of the pheromone, its geometric correspondence with the protein plays an important role. Therefore, the course of chemical reactions or a significant change in the initial geometry of the molecule will lead to the deactivation of the pheromone as a carrier of information.

The calculated characteristics of lepidoptera pheromones were compared with the data on the search activity of insects.

CONCLUSION

The efficiency of information transfer using pheromone molecules is determined by a number of factors, for example, such as the resistance of pheromones to the effects of the external environment on them, that is, to their physicochemical characteristics. The purpose of pheromones and the principle of their action is based on their preservation of their composition and structure, for a certain time, which should be sufficient for spreading in the air, and reaching individuals that must receive a chemical signal. And the use of highly resistant molecules as pheromones can lead to clogging of the information channel and disorientation of individuals receiving signals.

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