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METHOD FOR EXTRACTING PHYTONCIDES FROM PLANT MATERIAL

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TITLE. The article discusses biologically active substances from plants – phytoncides and their significance for humans. Methods for extracting some subclasses of phytoncides, in particular essential oils, saponins and glycosides, are presented. Scientific interest in these substances is due to the urgency of finding alternative ways to combat airborne pathogens, protozoa and insect pests that are safe for the environment.

KEY WORDS: phytoncides, fractions, essential oils, saponins, glycosides, plant material.

Phytoncides (from the Greek Φυτόν – “plant” and Latin Caedo – “I kill”) are biologically active substances secreted by plants that can destroy or suppress 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 designate a class of volatile substances produced by plants and having antimicrobial properties.

Phytoncides have a different chemical nature; in fact, they are 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, insecticidal activity, acting as an alternative to synthetic drugs with the same spectrum of action.

Extracting phytoncides from plant materials is a labor-intensive process. This is due, first of all, 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 look at the main methods.Essential oil extraction method. The most common method of extracting essential oils from plant materials is distillation. This method involves exposing the plant to water or steam.raw materials.

There are two types of distillation: direct distillation, in which the plant material is directly immersed in boiling water, and steam distillation, in which the raw material is placed on a screen over boiling water, allowing steam to pass through the raw material. In both methods, the glands of the plants are ruptured, releasing the essence they contain in the form of steam. This steam, together with the steam involved in the distillation process, is collected in a tube that passes through the cooling systems, after which it is returned to liquid form and enters the separator. The steam turns into a water distillate and the plant essence becomes an essential oil.

Coniferous plants, citrus fruits, herbs and abundantly flowering plants are the richest in essential oils.Saponin extraction method. Saponins are extracted from plant raw materials as follows: crushed dried raw materials are treated with petroleum ether to destroy complexes of saponins with sterols. The resulting material is then treated with 96% ethyl alcohol to obtain a total saponin extract. Saponins are precipitated from an alcohol solution using acetone. The resulting saponin fractions are purified by repeated precipitation to remove all impurities. Then 70% ethyl alcohol is added and centrifugation is carried out, resulting in diethyl dried powder for further study.

Sources of saponins can be plants such as horse chestnut, horsetail, St. John's wort, knotweed, chicory, marshmallow and others.Glycoside extraction method. Dried crushed plant materials are extracted with 96% ethyl alcohol. The alcohol extract is evaporated in vacuum to a thick residue, which is then dissolved in 35% ethyl alcohol and filtered through aluminum oxide (ratio of thick extract to adsorbent 1:5). The adsorbent is washed with 35% ethyl alcohol.

Glycosides are extracted three times from an alcohol-aqueous solution with 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 vacuo to obtain the purified sum of glycosides.

Glycosides are found in large quantities in the seeds of fruit plants, as well as in plants such as foxglove, belladonna, nightshade, etc.Both separately isolated fractions of phytoncides and their general combinations are of great interest to science, since it is in the format of a number of complementary biologically active substances that phytoncides provide natural immunity to plants.Insect pheromones, discovered more than fifty years ago, can now become a safe and harmless replacement for pesticides and other harmful chemicals that are currently used to control pests that cause enormous damage to agriculture.farm.

The question arises, what is the essence of pheromones? How do they act on insect pests? How they are influenced by external environmental factors, and most importantly, what are the prospects for using pheromones in agriculture.Pheromones are biologically active substances, products of external secretion released into the environment by insects, fish, animals, communication between organisms of the same species, a kind of volatile chemosignals that can control neuroendocrine behavioral reactions, developmental processes, social behavior and reproduction. Pheromones change the behavior, physiological and emotional state, and even the metabolism of different individuals of the same species. These substances, being means of regulation, play an important role in the communication of many species of insects, for example, ensuring the proximity of males and females during the breeding season, the concentration of insects on food plants and in wintering areas, 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. CurrentlyInsect pheromones are considered the most studied.There are two main types of pheromones, differing in their effects: releasers and primers. The first type is the transmission of danger signals between individuals of the same species. Usually releasers are people. Pheromones, by providing chemical releasers, are capable of inducing an individual to immediate action, for example, pheromones, highly volatile substances that spread through the air. The second type is the formation of special behavior and influence on other individuals, an example is pheromones secreted by the queen bee in order to suppress the sexual development of female bees, turning them into ordinary worker bees. Primers are most often distributed by contact. Currently, releasers have been studied better than primers; from 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 – repellent pheromones;primers designed to stimulate activity-inducing pheromones, such as anxiety pheromones; repellent, suppress reaction, etc. [5]The source of pheromone in insects can be individual secretory cells scattered throughout the body, or groups of them that form a special organ, the pheromone gland. The ducts of the pheromone glands open on the surface of the body or in cavities communicating with the external environment. Insects release pheromone in trace amounts: for example, the female codling moth (Cudiapomonella) releases only 9 nanograms of pheromone per hour. However, even this amount is enough for the male moth to smell and find the female in the crowntrees

Insects perceive the smell of pheromones using 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. In order for the insect to react, a very small amount of pheromone in the air is enough. [4]

Typically, pheromones are not one substance, but a mixture of the main, dominant component by weight with small additives (minor components): they can contain more than 10 components. One substance can have several different functions. Pheromone molecules are highly volatile and quickly disintegrate under the influence of atmospheric oxygen, moisture and light. According to their 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 insect pheromone, you can synthesize it in the laboratory. It is these synthetic analogues of sex and aggregation pheromones that can be used to protect plants from pests. The advantage of synthetic pheromones, which are used in microdoses, 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 pests.There are two main areas of use of synthetic pheromones against harmful insects: First, monitoring. This suggests that the use of pheromones makes it possible to record processes such as the flight of pests, obtain data on their numbers, or even the ability to determine the range of quarantine pests. Secondly, this makes it possible to prevent males from finding females, attract insects, and catch or destroy pests. By saturating the air with synthetic pheromone before they can find a natural source of the pheromone. In both cases, the reproduction of pests is blocked.However, in addition to the influence of pheromones on pests, it is necessary to take into account the influence of environmental factors on the pheromones themselves. Considering 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.Pheromone dispensers 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. Pipettes were renewed every 10 days. Observations were carried out over three years in fields of cotton, kenaf, corn, alfalfa, red pepper, tomato and pumpkin. Based on the number of males of each species caught in pheromone traps, indicators of the relative abundance of the species were calculated [4]. The number of moth complexes in cotton agrocenosescrop rotation

In the examined fields of cotton crop rotation (cotton, kenaf, corn, alfalfa), the bollworm complex, determined by the presence of pheromones, since differences were observed in individual years, is usually of the same type. As for mainly small species. Thus, in all areas the dominant species was bindweed (Emmeliatrabealis), the subdominant was the exclamation moth (Agrotisexclamationis) (Agrotissegetut). Corn moths (Spodopteraexigua) and corn moths and winter moths (Spodopterafrugiperda) were absent from corn and cotton fields. In a cotton field, bindweed (Emteliatrabealis), winter moth (Agrotissegetum), (Agrotisexclamationis), cotton bollworm (Helicoverpaarmigera), meadow moth (Mythimnaunipuncta), as well as gamma moth (Autographagatta), black bollworm (Hestia c-nigrum), upsilon bollworm (Agrotisipsilon). The species diversity of moths in the corn field was somewhat less exclamatory: there were no cotton bollworms (Helicoverpaarmigera) or epsilon moths (Agrotisipsilon).All cutworm species whose pheromones were used were identified in the alfalfa field. All cutworm species whose pheromones were used in the observations (Spodopteraexigua) and (Spodopterafrugiperda) were also found in vegetable fields. In the Tashkent region, the number of fall armyworms was generally the same, with the exception of the caradrina fall armyworm. On vegetable crops it was higher than on the fields of cotton crop rotation in the Yangiyul region. On vegetable crops, as well as in agrocenoses of cotton crop rotation, the dominant species was field bindweed (Emmelia trabealis), the subdominant (Agrotisexclamationis) was winter grass (Agrotissegetum). Thus, one day after installing pheromone traps, 14.7 individuals were caught on red pepper, and 11 individuals each on tomatoes and alfalfa. At the same time, in the fields of alfalfa, tomatoes and red peppers, 6, 7.7 and 10.7 individuals were found in traps with winter pheromones, respectively. According to available data, the generalized economic threshold of harmfulness is acceptedconsider catching on average per trap per day (night) (Agrotissegetum), which corresponds to a caterpillar density of 2.6 – 4.0 individuals per 1 m².

RESEARCH METHODS

Theoretical modeling and calculation of the physical characteristics of pheromone molecules were carried out within the framework of density functional theory using the BZLYP functional, using the basic packages 6-31G** and cc-pVDZ, implemented in the GAMESS-US program. Calculation of absorption spectra and optimization of molecules in the excited state were carried out using the TimeDeredent method. The influence of thermal effects on pheromone molecules was assessed using a method based on the calculation of normal vibration modes.Figure 1 – Atomic structure of lepidopteran pheromonesinsects

The geometry of lepidopteran insect pheromones allows the formation of conformers due to the rotation of parts of the molecule relative to each other around σ-bonds; therefore, at the first stage of the study, modeling and calculation of the properties of pheromones in various conformations other than the linear structure were carried out. For all conformers, the energy and structural characteristics in the ground and excited states were determined, 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 the molecules by no more than 24 kJ/mol, which is less than 0.001% of the total energy of the molecules. On average, the difference between the total energy of conformers is 6 kJ/mol. The slight difference in energy between the conformers indicates that pheromones have linear and twisted conformations, corresponding tothe absorption maximum with the corresponding values of the linear dimensions of the pheromone molecule, its electric dipole moment and length, corresponds to Lepidoptera insects; the formation of certain conformations is not typical. Maximum absorption. The influence of molecular geometry on the magnitude of the electric dipole moment and absorption spectra of pheromones is 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, the increase in dipole moment upon transition from linear to maximally twisted conformation is up to 50%.

Figure 3 – Atomic and electronic structure of pheromones in the ground and excited states: carbon atoms are indicated in blue, hydrogen atoms are in gray, oxygen atoms are in red.Exposure to electromagnetic radiation leads to changes in the electronic and atomic structure of pheromones. For disparlure, a pheromone of the gypsy moth (Lymantria dispar), which does not have multiple bonds in its structure, a change in electron density upon absorption of radiation occurs in the region of the epoxy ring for all conformers. Calculation of the geometry of the disparlure in the excited state shows similar structural changes for all conformations: the bond angles in the epoxy ring change, one of the C-O bonds increases on average to 0.9 A, which can subsequently lead to its rupture and, as a consequence, to ring opening and destruction of the pheromone. Most pheromones of lepidopteran insects belong to unsaturated compounds containing up to three double bonds in their structure. Analysis of the electronic structure of pheromone molecules shows that, regardless of the presence and type of oxygen-containing functionalgroup, the redistribution of electron density upon absorption of light occurs in the region of double bonds and corresponds to the transition of an electron from n-bonding to n*-bonding orbitals (Figure 3). Bond lengths are indicated in angstroms (A). Figure 3 shows the lengths of chemical bonds in molecules of unsaturated alcohol and unsaturated epoxide in the ground and excited states. The change in bond lengths occurs only in the region of multiple bonds and does not affect oxygen-containing functional groups. Similar structural changes occur in all unsaturated pheromones of Lepidoptera. The increase in double bond lengths occurs on average by 0.1 A. Such a change in bond length is unlikely to lead to the destruction of the original atomic structure of molecules only under the influence of electromagnetic radiation, but will increase their reactivity and will promote chemical reactions duringinteraction 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 to the protein plays an important role.Therefore, the occurrence of chemical reactions or a significant change in the original geometry of the molecule will lead to the deactivation of the pheromone as an information carrier.The calculated characteristics of lepidopteran pheromones were compared with data on search activityinsects

CONCLUSION

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

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