Institute of Chemistry, Republic of Moldova
Institute of Microbiology and Biotechnology, Republic of Moldova
Institute of Genetics, Physiology and Plant Protection, Republic of Moldova
Tiraspol State University, Republic of Moldova

E. COROPCEANU, A. CILOCI, A. ŞTEFÎRŢĂ, I. BULHAC

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Coropceanu E., Ciloci A., Ştefîrţă A., Bulhac I.

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Aluchi Nicolae – PhD, Tiraspol State University
Codreanu Svetlana – PhD, Institute of Microbiology and Biotechnology

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ABBREVIATIONS

acac – acetylacetonate
Ala – alanine
An – aniline
APX – ascorbate peroxidase
Arg – arginine
Asp – asparagine
bpe – 1,2-bis-(4-pyridyl)-ethane
bpy – 4,4′-bipyridyl
CAT – catalase
CC – coordination compound
CK – cytokinins
CL – cultivation liquid
CO – cytochrome oxidase
Cys – cysteine
Dena – N,N-diethylnicotinamide
DfH_2 – α-benzildioxime
$DioxH_2$ – α-dioxime
DH_2 – dimethylglyoxime
DMSO – dimethylsulfoxide
DNA – deoxyribonucleic acid
EAA – efficiency of assimilating apparatus
EDDS – ethylenediamine disuccinates
EEiNA – ethyl ester of isonicotinic acid
EENA – ethyl ester of nicotinic acid
Hs – hydric stress action
G – growth index
Glu – glutamine
Gly – glycine

GPX – glutathione peroxidase
GR – glutathione reductase
IAA – indolyl acetic acid
Inia – isonicotinamide
Ile – isoleucine
Leu – leucine
LPO – lipid peroxidic oxidation
Lys – lysine
MDA – malondialdehyde
Met – methionine
MH_2 – methylglyoxime
NADP – nicotinamide adenine dinucleotide phosphate
Nia – nicotinamide
$NioxH_2$ – 1,2-cyclohexandione dioxime
PA – pectolytic activity
PhAS – physiologically active substances
Phe – phenylalanine
Pro – proline
PVP – polyvinylpyrrolidone
PX – peroxidase activity
Py – pyridine
RNA – ribonucleic acid
ROS – reactive oxygen species
RSP – readily soluble proteins
SAM – sulphanilamides
Sam – sulphanilamide
SD – saturation deficit
Seu – selenourea

Ser	–	serine
SOD	–	superoxide dismutase
Thio	–	thiourea
Thr	–	threonine
TWC	–	total water capacity
Trp	–	tryptophan
Tyr	–	tyrosine
Val	–	valine
WUE	–	water use efficiency

FOREWORD

Contemporary society is a consumer of technologies, that need permanent modernization/updating, in a more and more dynamic rhythm. New phenomena and facts explained by scientists determine the evolution of knowledge and advanced technology. In this context, contemporary research is directed, on the one side, toward practical, interdisciplinary and useful for economy investigation and, on the other side, toward the implementation of new achievements in the process of instruction, so as to connect the two reciprocally dependent domains: research and instruction. Subsequently, the development of pupils and students' research competency is a key element to their innovational competency formation, that is, in its turn, the engine of society development.

Coordination chemistry represents an intersection field between inorganic and organic chemistry, generating compounds with properties absolutely different from mineral salts and initial organic ligands. Oriented synthesis allows the attainment of certain molecules with programmed qualities (composition, structure, properties) that lead to the attainment of molecules with vital biological importance (vitamin B_{12}, hemoglobin chlorophyll etc.) and their analogs, superior to them by some properties. The use of coordination compounds in agriculture, medicine, industry is a process of development that brings profitability and economic benefits.

The results described in the present work reflect the experience of over 20 years of collaboration among the laboratories of Coordination Chemistry (Institute of Chemistry), Enzymology (Institute of Microbiology and Biotechnology), Plants Nutrition and Water Regime (Institute of Genetics, Physiology and Plant Protection) of the Academy of Sciences of Moldova and the Departament of Chemistry of Tiraspol State University (from Chișinău). The work is recommended for the students of the specialties of Biology and Chemistry etc.

The authors would like to thank all people that contributed to the achievement of this research.

Chapter I.

AN OVERVIEW OF THE PERSPECTIVES OF USING COORDINATION COMPOUNDS BASED ON DIOXIME LIGANDS

The synthesis of coordination compounds that manifest useful properties, such as the capacity of adsorbing small molecules into intermolecular cavities [1-4], luminescent manifestation [5, 6] catalyst [7-9], and sensor properties [10], influence of certain physiological processes in living organisms [11-13] etc., create real premises for effective intercalation of research and production fields. The attainment of materials with porous properties [1, 2] based on the compounds of some transition metals indicates on the possibility of assembling networks with a different degree of porosity depending on the nature of the ligands.

Identifying the domains of practical application of new chemical compounds is one of the most important objectives of contemporary science focused on knowledge transfer from the research domain to that of modern technologies development based on new achievements. Coordination compounds of transition metals with chelate ligands, that include the dioximates of transition metals, play an important role in contemporary chemistry. The capacity of α-dioximes to complex with *d*-type metals draws researchers' attention not only from the perspective of synthesizing the models of such natural compounds like B_{12} or hemoglobin [14], but also due to the broad spectrum of synthetic, analytical and structural possibilities. Dioximates can be used as catalysts of industrial processes [15], antihypoxic or antidote preparations [16, 17] for separating and purifying the metals that generate complexes etc. An important contribution to the synthesis and study of some transition metal dioximates was made by the school of Chişinău, founded by the famous chemist, academician A. Ablov. After studying the behavior of some representatives of this series in solution, it was assumed that, due to its high stability, the equatorial fragment $Co(DioxH)_2$ (DioxH – dioxime monoanion) doesn't modify, but there can devolve reactions of substituting the ligands from positions 1,6 [18].

The history of synthesizing coordination compounds based on oxime ligands, namely dioxime, begins in 1905, when the Russian chemist L.A. Chugaev, discovered the capacity of α-dioximates to form compounds with a series of transition metals: Ni, Pt, Pd, Fe, Co [19].

Subsequently oxime ligands were well described in the literature [20, 21]. Further research on transition metal dioximates were continued by different research groups from coordination chemistry domain, the most famous being those headed by Y. Nakatsuka and H. Iinuma [22], Ablov A.V. [23] etc. The presence of nitrogen and oxygen atoms as potential electron donors in oxime groups favors the coordination of dioximes with transition metals within coordination compounds. In complex combinations, α-dioximes usually play the role of chelating bidentate ligand. Coordination by means of nitrogen atoms, rather than by means of oxygen, can be explained, in the first case, through the formation of five atom cycles that, according to L.A. Chugaev, are more stable than those of seven atoms, that would form at dioxime coordination by means of oxygen atoms. It is considered that the stability of $Me(DioxH)_2$ planar fragment is influenced by the formation of two quasi-aromatic cycles within which take place metal-ligand π–dative interactions [24], and the two intramolecular hydrogen bonds formed among oxime groups of dioxime monoanions increase the stability of this fragment (Fig. I.1).

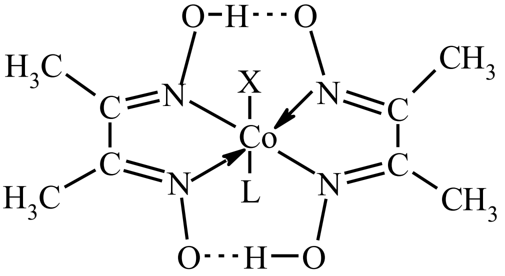

Figure I.1. Structure of cobalt(III) *trans*-dimethylglioximates.

This explains the fact that out of those three isomers, only *anti*-isomers (*E*,*E*) form stable complexes with metals. If on the *trans*-coordinate there are two different ligands (L and X), than the Co(III) atom forms a π bond with the ligand with a higher donor (X) capacity. In this case, it can be noticed the weakening of Co-L bond.

Due to their high stability, transition metals *trans*-dioximates can be hardly exposed to isomerization and substitution of dioximate radicals from the internal sphere of coordination, being stable in acid, neutral and weak basic media.

The increased stability of the equatorial fragment of Co(III) *trans*-dioximates permits to study the substitution reactions on L-Co-X axial coordinate [25]. Despite the high stability of $Co(DH)_2$ group, the literature in the field mentions cases of dioxime ligands substitution [26]. This phenomenon takes place both under the action of salicylaldehyde thiosemicarbazone [27] and of adipoid selenosemicarbazone [28].

The study of the interaction of some 3*d* type metal cations with α-dioximes in an acid medium made possible the development of the general method of synthesis, in a strong acid medium, of some new classes of transition metals coordination compounds with α-dioximes: *tris*-dioximines with the general formula $[M(DioxH_2)_3]X_2$ (M – Fe^{2+}, Ni^{2+}, Cu^{2+}; $DioxH_2$ – non-deprotonated molecules of dimethylglioxime (DH_2) and 1,2-cyclohexanedionedioxime ($NioxH_2$); X – Cl^-, Br^-, NO_3^-, ClO_4^-, ½SO_4^{2-}, ½$S_2O_3^{2-}$), and of their structural analogues – *cis*-dioximines with the general formula $[X_2M(DioxH_2)_2]$ (M – Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}; X – Cl^-, Br^-, ½SO_4^{2-}, ½$C_2O_4^{2-}$) [29]. By using physical methods of research (magnetochemistry, NMR, IR, UV-Vis, X-ray and electronic, and Mössbauer spectroscopy, and single crystal X-rays diffraction method), it was established the stereo-chemistry of the obtained compounds and it was demonstrated the new structure type in the wide series of transition metals coordination compounds based on α-dioxime [30].

The variety of dioximate composition and structure is due to different synthesis conditions (pH of solution, reaction temperature etc.), nature of axial ligands and anions from the external sphere and of other factors. One of the promising directions of using dioximates is the research of catalyst properties of metal coordination compounds in different physiological and industrial chemical processes. As a result of these researches, it was assumed that some dioximates can be used as catalyst in the process of dyeing fabric. They were proposed for use to the enterprises of Kalininsk, Iarţev, Ternopol and Tiraspol [31]. Cobalt(III) dioximate with nicotinamide was experimentally tested in the process of treating hemolytic anemia and other diseases [32].

The diversity of transition metal dioximates is present in mono-, poly-, homo- and heteronuclear compounds, with di- and polymeric structure.

Mononuclear dioximates are well known and represent compounds with a high stability, offered by pseudo-metal cycles formed through the coordination of dioxime monoanions in the octahedron equatorial plane, cimented by means of intramolecular hydrogen bonds among oxime anions. Later, it was achieved the synthesis of different types of heteronuclear dioximates [33], fact showing the diverse capacity of coordination affinity of transition metals towards the nitrogen or oxygen atom of oxime groups. A special interest in the domain of molecular design and attainment of complexes with a varied composition represent the *tris*- and *cis*-dioximines of some transition metals [34], and their clatrato chelates [35]. The latter can be obtained by joining boron(III), tin(IV), germanium(IV) compounds to three bideprotonated radicals of dioximes, and through other attempts to diversify the composition and architecture of representatives of this series of complexes.

The transition from dioximates with monomeric structure to those with di- and polymeric structure is carried out by using different bridging-ligands binding the atoms that generate complexes among themselves [36-41]. The synthesis of new dioximes with different functional groups, that offer new possibilities for molecular composition and structural architecture, is one of the perspective directions of coordination chemistry of oxime development [42].

Cobalt (III) dioximates containing the fluorine atom differ essentially by their structure from traditional *bis*-dioximates. Within them, there achieve bi- and trinuclear associates in the ratio Co:DH=1:2, where α-dioxime units can be found in the same plane or in reciprocally perpendicular planes in the metal coordination polyhedron.

The given synthesis reactions were carried out in the traditional way, explored at dioximate synthesis.

Thus, at the interaction of cobalt fluorine $CoF_2·4H_2O$ with dimethylglyoxime, in the presence of hydrofluoric acid and hydrogen peroxide, there were obtained crystals of $[Co_2(DH)_4O_2(H_2O)_2]·2HF·2/3H_2O$. According to the data of EPR and NMR spectra the compound shows a *trans*-configuration, the cobalt atom has the oxidation degree +3, while the fluorine atom can be found in the external sphere.

Crystalline structure deciphering proved that the neutral complex $[Co_2(DH)_4O_2(H_2O)_2]^0$ consists of $[Co(DH)_2H_2O]^+$ cations, bound through O_2^{2-} bi-atomic ion, HF and molecules of crystallization water [43]. The complex is binuclear, while the bridge fragment Co-O-O-Co is liniar (Fig. I.2), HF and water molecules play the role of particles from the external sphere.

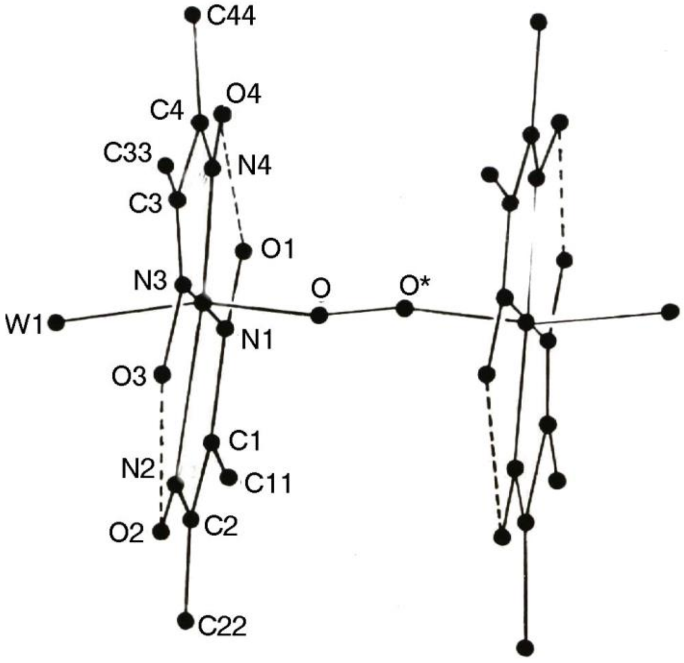

Fig. I.2. Dimer fragment of $[Co_2O_2(DH)_4(H_2O)_2]·2HF·2/3H_2O$ structure.

Further, from $CoF_2·H_2O$ – DH_2 – HF system, it was obtained the trinuclear complex with mixed valence $[Co_2^{III}Co^{II}(DH)_6]F_2·8H_2O$ (Fig. I.3).

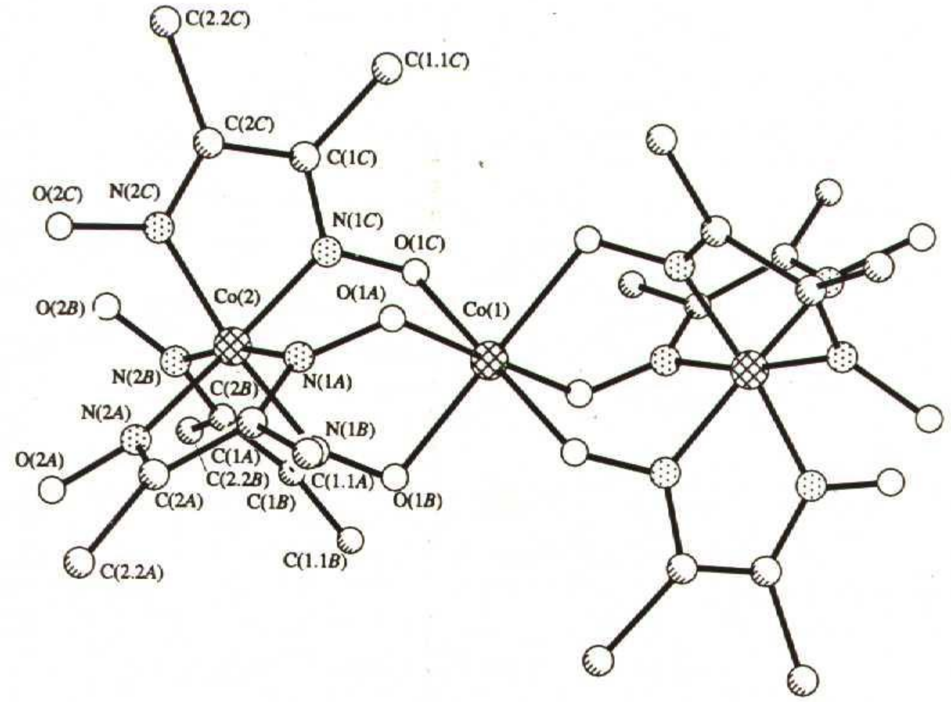

Fig. I.3. Structure of $[Co_2^{III}Co^{II}(DH)_6]^{2+}$ trinuclear centrosymmetric cation.

The radicals of dimethylglyoxime are monodeprotonated, binding to a cobalt atom by means of two nitrogen atoms, and to the other – by means of the deprotonated oxime group, as in the *„cis”*-binuclear dioximates [44]. Two cobalt atoms of the complex have the oxidation degree +3, and one +2. Co(III) atom, surrounded by six nitrogen atoms, has an octahedral configuration, achieved on the account of three units coordinated by mono deprotonated dimethylglyoxime. Co(II) atom is surrounded by six oxygen atoms of deprotonated oxyme groups of dimethylglyoxime units. Two fluorine atoms with basic function stabilize the complex structure by means of intramolecular hydrogen bonds.

As researches had proved that certain dioximates can show properties of biochemical processes stimulators, and catalysts within industrial processes, it presents a special interest the establishment of new potential domains of using the representatives of this class of coordination compounds.

From the point of view of practical application of transition metal dioximates, currently, there appear many directions where the representatives of this class can be successfully used, for instance: in the synthesis of artificial models of certain vital biologic molecules; in the elaboration of biotechnologies for attaining (through oriented synthesis) microbial enzymes widely used in different areas of food and pharmaceutical industries; in the attainment of certain compounds with useful properties for medicine; in the synthesis of materials with valuable physical properties (semiconductors, photoluminescent and dielectric materials); in the attainment of systems used in hydrogen production etc.

The use of coordination compounds as stimulators of different organisms' biologic activity is one of the most solicited directions for solving various problems connected to medicine, food industry, agriculture etc. Modern biotechnology pays a special attention to the oriented synthesis of bioactive substances by microorganisms. They are known as economically advantageous sources of a large range of valuable bioactive substances attainment [45].

A class of biologically active substances of a great importance for biology, with multiple practical applications, represent the enzymes. Enzymatic preparations are extensively used in the most diverse industries, such as medicine and agriculture. A special attention is given to the enzymes of microbial provenance, due to the wide possibilities of their use in solving practical problems of biotechnological productions. A major importance for industry and medicine have the hydrolases synthesized by fungi. Hydrolases are enzymes that catalyze hydrolysis reactions of natural polymers with a large molecular weight, that stay at the basis of many modern technological processes.

Glycosidases of mycological nature effectively hydrolyze plant polysaccharides such as cellulases, hemicellulases, pectin, starch that do not assimilate in the human and animal digestive tract. Glycosidases synthesized by micromycetes of *Trichoderma, Aspergillus, Mucor, Penicillium* genus are used in food and light industry, and in the process of fodder production. An essential practical value have the proteases synthesized by micromycetes. Serine proteases produced by the fungi representing *Acremonium, Alternaria, Aspergillus, Fusarium, Penicillium, Trichoderma* genus break ester bonds and those formed by the radicals of leucine and methionine aromatic amino acids. Acid proteases synthesized by micromycetes of *Aspergillus, Trichoderma, Penucillium, Rhizopus* genus hydrolyze peptide bonds formed by aromatic amino acids and other hydrophobe aminoacids and are able of hydrolyzing casein, hemoglobin, bovine serum protein, gelatin and fibrin.

The enzyme role, without alternative, in creating and developing biotechnologies increases the interest towards microscopic fungi as potential producers of extracellular enzymes. Thus, microscopic fungi continue to remain perspective objects for modern biotechnologies. Remarkable by the broad range of enzymes and the short development cycle, the possibility of oriented synthesis of secondary metabolites confers additional technological importance to micromycetes. The numerous practical implications of hydrolytic enzymes (pectinases, amylase, cellulase, protease, lipase) in different economic and social spheres, determines the necessity of elaborating certain effective proceedings aimed at increasing and maintaining the biosynthetic capacity of microorganism producer. From this point of view, it presents interest the use of chemical stimulators, especially of transition metal coordination compounds. Complex structure and composition, metal presence as a central atom. confirm the perspective of using coordination compounds as stimulators and regulators of biologic processes in the microbial cell.

In this context, the study of the biological activity of transition metal coordination compounds becomes an actual problem, due to their massive practical implications in medicine and biology, and the possibility to serve as a theoretical support for the synthesis of substances with programmed properties [46-56].

Spontaneous morphological, physiological and synthetic modifications typical of fungi and other microorganisms as a responsive (adaptation) reaction to the change of external conditions of medium create an excellent platform for orienting the synthesis of enzymes in mycelia fungi with the use of coordination compounds as a modifying factor of the nutrient medium [57, 58].

The biological properties of metal complexes can be mostly attributed to the presence in their composition of Fe, Cu, Mo, Co, Zn, Mn, Ni etc. metals, that represent microelements bound to atoms and groups of atoms from molecules of organic substances. These elements, entering in very small amounts in cells composition, play an important role in their vital activity. The many-sided influence of microelements is important due to their participation in the fine reactions of substance cell exchange, especially in fermentation processes. Copper, zinc, cobalt, manganese, molybdenum, calcium and iron participate in the processes of oxidation-reduction, that take place in vegetal and animal organisms, are components of a series of important oxidising ferments, participate in the glucide and proteic metabolism in organisms. Microelements, especially biometals like cobalt, copper, zinc and manganese, participate in the formation or activation of ferments, vitamins, hormones; regulate the exchange of substances, determining the growth, development, reproduction, productivity and production quality.

Metal ions fulfil a lot of functions: they represent an electrophilic group of ferment active center that facilitate interaction with negative charge sectors of substrate molecules; form a catalytically active conformation of some molecule structure (zinc and manganese ions participate in the formation of the spiral structure of the RNA); participate in the transportation of electrons etc.

Metal ion capacity to form the ferment active center depends on the metal coordination capacity, geometry and stability of the formed complex. An important role in manifesting the properties of metal complexes play the ligands from their composition that, due to the wide set of electron donor atoms, create with transition metal ions stable complexes, different as composition, structure and characteristics. Biologically active substances included as ligands in the composition of metal complexes essentially increase their efficiency. Coordinatively bound metals are less toxic and have a higher reagent capacity [59-65].

The synthesis of complexes that present models of biological objects and their action testing on the natural metabolic processes of microorganisms is a promising direction of reproduction and natural biosynthesis control. According to their structure, metal complexes resemble to biological natural complexes, regulating the vital functions of microorganisms (chlorophyll, hemoglobin, vitamin B_{12}, insulin etc.). It should be mentioned that hemoglobin (central atom – Fe^{2+}), chlorophyll (central atom – Mg^{2+}), vitamin B_{12} (central atom – Co^{2+}), insulin (central atom – Zn^{2+}) are metal complexes. Nature itself evaluated the value of macromolecular biomolecules in achieving biological functions of vital importance for microorganisms [66]. In most metal complexes, the atoms generating complexes are the biometals, whose action is bound to proteins, specific ferments. Metal ions play an important role in enzymatic catalysis processes. The ligands from the composition of coordinative compounds can present molecules with biological properties etc.

As it has been established, coordination compounds containing cobalt can serve as stimulators of micromycete hydrolytic ferments biosynthesis [67-73]. A special interest presents the study of the influence of complex compounds of this class on microalgae. After a series of biological testings, where Co(III) dioximates containing fluorine were introduced in the nutrient medium of microorganisms, it has been found that they manifest properties that stimulate the biosynthesis of vitamin B_{12} in *Spirulina platensis* alga. The formation of compounds with cobalt diminishes the toxic action of fluorine ions present in the nutrient medium [74]. The inclusion of $[Co_2(DH)_4O_2(H_2O)_2]\cdot 2HF\cdot 2/3H_2O$ complex in the nutrient medium increased the quantity of vitamin B_{12} within spirulina biomass by 30.76-66.66% as compared to $Co(NO_3)_2\cdot 6H_2O$, and by 18.60-51.16% in comparison with cu $[Co(DH)_2(H_2O)_2]NO_3$. These studies created premises for initiating syntheses aimed at developing synthetic analogs of natural systems, modeling biologic molecules and analyzing their influence on the processes taking place within the cell.

Analysis of literature data showed that the biomass of *Spirulina platensis,* obtained through cultivation in the presence of cobalt coordination compounds, is characterized by a high content of cyanocobalamin, protein, carotenoids, and a balanced content of other components [75]. It has also been found that cobalt compounds with fluorine possess stimulating properties on some microorganisms. The complexity of structure and composition, and metal presence as central atom confirm the perspective of using coordination compounds as stimulators and regulators of biologic processes within the microbial cell.

Porphyridium cruentum red microalga is a well-known research object of phycology. In biotechnology *Porphyridium cruentum* is used as a producer of polyeneic fatty acids, phycobiliproteins and sulfated polysaccharides [76]. The recognized technologies of cultivating microalgae include techniques of using organic and anorganic stimulators of biosynthetic activity and growth. In this context, there are developed technologies of cultivating microalgae and cyanobacteria where metal coordination compounds are used as stimulators of biosynthetic processes [77]. *Porphyridium cruentum,* being an eukaryotic organism, developed systems and mechanisms of resistance to the presence of xenobiotics in the cultivation medium and, depending on the metal type and its way of complexing, it is obtained an effect of selective stimulation of biologically active substances in the algal biomass [78].

There have been achieved a series of experiments focused on the study of the influence of coordination compounds based on dioxime ligands on *Porphyridium cruentum* microalga. At the introduction of the complex of *bis*-(dimethylglyoximato)chloro(isonicotinoylhydrazone-2-hydroxy-1-naphthaldehyde)cobalt(III) in the nutrient medium, it increased the content of lipids and eicosapentaenoic acid in the dry and absolute biomass of microalga [79].

One of the biological effects of coordination compounds on microorganisms is their action on the growth process, that is why, one of the main indicators of microalgae tolerance to xenobiotics from the medium is the test of biomass accumulation. At the introduction of $[Co_2Cl_2(DH)_4HL]$(HL – 3-pyridinecarbaldehyde-isonicotinoylhydrazone) complex in the nutrient medium of *Porphyridium cruentum* microalga, the lipid content in biomass increased by 23.6-25%, while the eicosapentaenoic acid content increased 5.15-5.25 times [80]. The biomass of *Porphyridium cruentum* microalga represents a source of omega-3 fatty acid and eicosapentaenoic acid, fact that increases the interest for intensifying these physiological processes.

The introduction of $[Co(DH)_2(Thio)_2]_2[TiF_6]\cdot 2H_2O$ complex in the cultivation medium stimulated the productivity of *Porphyridium cruentum* microalga by 20% and lipogenesis by 17%. The compounds $[Co(DH)_2(Thio)_2]_2F[PF_6]$ and $[Co(DH)_2(Thio)_2]_2[TiF_6]\cdot 2H_2O$ stimulated the synthesis of proteins by 64-80% [81]. Protein accumulation is an indicator of microalgae biosynthetic activity.

The high activity of metal complexes can be explained by the energetic and conformational state of molecule tension, conditioned by space geometry, specific system of chemical bond lengths, coordination number etc. [82].

Cellular macrobicylic complexes with encapsulated metal ion (clathrochelated) represent a relatively new class of compounds possessing important physical and chemical properties. It has been achieved the synthesis of a series of clathrochelated *tris*-dioximates as potential preparations in cancer therapy. Selfassembling reaction of clathrochelates and their interaction with nucleic acids can be widely used in immunology and molecular biology [83-85].

Specialty literature has recently published a series of works describing the possibilities of using transition metal dioximates in the processes of water decomposition within artificial photosynthesis [86]. Cobalt(III) dioximates can be used in the process of electrocatalytical attainment of hydrogen [87-92]. Cobaloximes represent one of the most successful class of synthetic compounds of transition metals known in hydrogen production, that can be easily synthesized, are stable towards oxygen, can couple in artificial and natural photosynthetic systems [93, 94].

In contemporary agricultural practice, to render efficient plant cultivation, different fertilizers are used to facilitate genetic potential achievement with a higher outturn, to prolong fructification period or to reduce losses in cases of environment conditions aggravation [95, 96].

In the last decades, it increased the interest towards metal coordination compounds, especially towards those containing micro- and/or macroelements, with a high biological activity, that determine their wide use in agriculture, medicine and zootechny. Physiologically active substances (PhAS), having at their basis transition metal coordination compounds, have a great perspective as concerns their use in increasing crop plant resistance to adverse environmental conditions, especially to drought, suboptimal temperature conditions, deseases and vermins, thus contributing to the increase of plant productivity, and to crop quality melioration [95].

A growing interest has been lately granted to complex compounds of metals with antichlorotic properties, properties of biocatalyzers and stabilizers of metal oxidation degree, and properties of transporters of important physiological cations through cell membranes [96-99]. Complex compounds containing elements of mineral nutrition, unlike mineral fertilizers, are more effective and can be used in significantly smaller concentrations [100, 101]. Coordination compounds penetrate more easily in vegetal cells, are more labile, less toxic and more effective as compared to the ions of inorganic salts metals [102-105]. Therefore, it presents a great interest the study of coordination compounds effect on physiological processes, growth, development and productivity of some crop plants in adverse conditions of humidity and temperature. Using different proceedings, there have been synthesized a series of dioximates, whose composition, structure and physico-chemical properties were determined by means of modern research methods.

The synthesized compounds have been exposed to a series of tests aimed at checking their capacity of influencing physiological processes in some microorganisms and crop superior plants.

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Chapter II.

THE INFLUENCE OF DIOXIME LIGANDS BASED COMPLEXES ON THE BIOLOGICAL ACTIVITY OF SOME ENZYME-PRODUCING FUNGI STRAINS

In recent years, the potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzimatic activity in several microorganisms [1-4]. Among them, microscopic fungi distinguish by their genetically rich potential of synthesizing enzymes [5-7].

II.1. Mycelium fungi: sources of extracellular hydrolases

Fungi represent important study objects for fundamental biological sciences. Eukaryotic organization of their genetic apparatus, high adaptability to environmental conditions and metabolic plasticity place mycelium fungi on the first position as model objects involved in the development of such cardinal scientific directions like genetic structure study and regulation of genes activity, genetic control of translational, reparation and recombination processes. They underly the elucidation of molecular mechanisms of mutations, termophily, osmophily, and organism biochemical adaptability to environmental changing conditions and stressors [8-15].

An essential role in biotechnology development devolves to micromycetes. The broad spectrum of synthesized enzymes, the low prime cost of technologies and the easiness of obtaining microbial enzyme preparations, as compared to enzymes of vegetal and animal origin, render microscopic fungi effective objects of modern biotechnologies. Scientific data gathered so far show that namely fungi, due to their diverse range of physico-chemical characteristics, may become main producers of biotechnology, substituting plants, animals, and bacteria [5, 6, 16, 17].

The high technological qualities of imperfect fungi, ensuring priority over other organisms, are: short development cycle (2-10 days), adaptive metabolism, unusual intensity of cell synthesis, assimilation of a wide range of different substrates presented in most cases of by products and waste of different productions and agriculture, high yields and final product purity. The ability of micromycetes to secrete enzymes in the culture medium attributes them additional biotechnological value [18, 19].

In trade, fungi have been recently efficiently involved in the production of antibiotics, vitamins, antioxidants, hormones, aromatizers and food dyes, lipids, and polysaccharides. Their implications are massive in heavy manufacturing, especially in the attainment of organic acids, fodder amino acids, proteins and fuels. Secondary metabolites of mycelium fungi play an important role in medicine, pharmaceutical industry and food industry, and represent significant economic factors in society development [20-24]. Additional to the multiple conventional fermentation processes, mycelium fungi are applied in the processes of different organic substances biotransformations, constituting the essence of fine chemical industry in producing mono isomers and intermediate compounds inaccessible to chemical synthesis (attainment of new terpenoid substances from sclareol, microbial biotransformation of steroids) [8, 25-29]. Fungal biotechnology has produced a real revolution in enzyme technology. In addition to bacteria, mycelial fungi constitute the basic potential of fermentation industry [2, 8, 10-12, 23, 27, 30-32].

Out of the great diversity of metabolites, with a complex chemical structure and specific biological characteristics, synthesized by microorganisms, enzymes distinguish due to their significant role in life biology and diversity of practical applications. Enzymes are directly used as final products (medicinal remedy, enzymatic electrodes, indicators, analytical reagents, biosensors) or as agents in creating and improving various biotechnologies, particularly for processing plant and animal material, in the processes of chemicals fine transformation in order to assign specific properties (conversion of arachidonic acid into prostaglandins, penicillin into 6-aminopenicillanic acid) etc. [19, 33-37].

The significant catalytic properties of enzymes to mediate, in moderate conditions of pH, temperature and pressure, the multitude of complex and diverse biochemical reactions, the high substrate specificity, without equivalent in chemical catalysis, considerably influenced the extent of their application field. Enzyme preparations are widely used in agriculture, food industry, chemical and pharmaceutical industry, textile industry, paper and detergents production industry, in leather and fur processing, in obtaining extractive substances, in producing concentrated feed and coarse fodder uplifting. Highly purified enzymes are used in medicine and scientific research, in electronics, as components of cosmetics and food supplements [8, 10, 19, 27, 33-35, 38-40].

It is highly appreciated the contribution of enzymes in biotechnology development. The use of enzymes in various fields determines the improvement of technologic processes, medical diagnosis and analytical research methods; the efficient use of raw materials; the creation of low environmental risk biotechnologies, subsequently ensuring higher indices and increased yields in industry and agriculture. The production of enzymes is one of the most mobile and perspective branches of biotechnology, representing, at the same time, the strategic branch that determines modern biotechnology development.

Microbial enzymes derived from bacteria, molds, yeasts are, particularly, the most widely used. They acquired a significant value due to their economic and technical advantages, and are, therefore, firmly substituting the enzymes of plant and animal origin within different processes [8, 27, 40].

The major mass of enzyme preparations involved in industries is represented by hydrolases (pectinases, cellulases, proteases, amylases, and lipases), known also as industrial enzymes due to their broad application in various technological processes of natural polymers degradation into simple substances that serve as raw materials for many productions. Namely hydrolysis constitutes the basis of modern technologies of processing plant matter.

Important producers of hydrolases are the micromycetes, noteworthy for their unique spectrum of synthesized enzymes [5, 6]. The value of imperfect fungi, as producers of enzymes, consists in their ability to synthesize enzyme complexes. While processing multicomponent substrates, priority is given to enzyme complexes stemming from a single producer strain, due to the superiority they manifested. The enzymatic components of these complexes are more balanced as related to physico-chemical properties of their action mechanism, possess high stability, exhibit a more obvious catalytic synergism, that ensuring a deeper hydrolysis of natural substrates with increased economic indices [33-36, 40]. The respresentatives of *Aspergillus, Alternaria, Botrytis, Fusarium, Penicillium, Rhizopus, Mucor, Sporotrichum, Trichothecium, Sclerotinia, Chaetomium* and *Trichoderma* genera [19, 39, 41-48] distinguish by a high frequency of extracellular hydrolase biosynthesis.

The high responsiveness of mycelium fungi to varying external environment, commutative metabolism and induced character of synthesized hydrolases create an excellent platform for controlling the processes of enzyme biosynthesis by modifying the nutrition factor, the cultivation parameters, the application of specific inducers and influencing physical and chemical agents [49, 50].

The problem that face the productions using fungi is connected to the increase and stabilization of producer strain biosynthetic capacity caused by the spontaneous variability specific of microorganisms. The continuous application of programs for preserving and enhancing the biosynthetic potential of producer microorganism strains is an essential element in improving industrial processes aimed at overcoming the economic barrier. The studies concentrated on regulation and stimulation of enzyme biosynthesis at cellular level are of particular importance in establishing the regularities of ferment formation under the action of influencing external factors, in determining new ways of controlling and directing synthesis, and play a particular part in creating progressive biotechnology [51-57].

The continuous expansion of enzyme application field confirms the perspective of research in intensifying the biosynthetic capacity of producing microorganisms. In this aspect, a new perspective is open by coordination compounds of transition metals. The structural and composition complexity, the presence of metals as central atoms, the wide range of organic ligands fit perfectly the prospect of using coordination compounds as stimulators and regulators of biosynthesis and enzyme activity in microorganisms.

In biological terms, the perspective of using coordination compounds is primarily attested by the fact that, in their composition, as complex generating atoms are incorporated the metals with the status of trace elements: Co, Cu, Zn, Mn, Fe, Mo, Mn, Ni et al. Trace elements are significant for the vital activity of organisms. Being components of proteins, enzymes, vitamins and hormones, they actively participate in regulating important biochemical processes taking place in living cells [58-60]. The stimulating action of most of them had been detected long ago [61]. However, their inorganic compounds, such as mineral salts, due to their high toxicity, haven't been explored yet neither in medicine nor in agriculture. More prospective seems to be the use of metal coordination compounds. Coordinately bound metals are less toxic and possess a higher reaction activity [59, 56].

It's known the important role of metalocomplexes for biological objects as agents intensifying plant productivity, development and growth, and resistence to adverse natural conditions; strengthening specific immunity in animals; increasing the productivity of biotechnologically explored algae and cyanobacteria, and of some representatives of other taxa - fungi, bacteria, yeasts, actinomycetes [55, 61-77].

Specialists in coordinative and bioinorganic chemistry explain the high activity of coordination compounds through the tense conformational energy state of metalocomplex molecule, conditioned by spatial geometry and coordination number etc. [58, 69, 78].

Recent research convincingly demonstrates the possibility of optimizing and regulating the synthesis of microbial metabolites by means of transition metal coordination compounds [79-98], highlighting the important role of coordination compounds in orienting microbial synthesis.

Promising results have been obtained in research on bio-stimulating and regulating effect of metal coordination compounds on the biosynthesis of exocellular hydrolase in mycelial fungi, an area with great innovational, fundamental and applicative potential [72, 90, 99-113].

Metal ions play a special role in enzymology. A large number of metals are incorporated into certain enzymes, forming metal enzymes, while other metals ions serve as activators of enzymes and enzyme systems, participating in a wide variety of processes. About 1/4 to 1/3 of studied enzymes are metal enzymes needing at least a metal ion as a functional component. They include metal ions, particularly of *d* type, that being stereochemically bound in the structure of enzymes, influence their catalytic function by fixing the substrate or the co-factor, by activating the enzyme-substrate intermediary complex, by stabilizing the apoenzyme conformation, by modifying the enzyme spatial structure, and by metal ion direct participation in redox reactions within enzymatic reaction [32, 53, 55, 56, 58, 114].

Examples of metalloenzymes can be considered the proteins containing ions of iron, zinc, copper or bismuth in their active center. The enzymes with zinc were identified in all the 6 classes of enzymes: oxido-reductase, transferases, hydrolases, lyases, isomerases, ligases. Catalases and many peroxidases are typical heme enzymes that require iron. Superoxide dismutase metalloenzyme, isolated from eukaryotic citoplazma, contains two atoms of copper and two atoms of zinc. The metals Mn, Mo, Co, Ni were identified as parts of matalyoenzymes [56, 58, 78, 114]. Chlorophyll, heme, cytochromes, myoglobin, vitamin B_{12}, and metal-polynucleotides represent natural complexes of metals with proteins and nucleic acids [115].

Metals-activators, though weakly associated with protein, by forming easily dissociable metal-enzyme complexes, become essential for enzyme activity, thus prompting the enzyme transition from the zymogen inactive state to the catalytically active state [44, 56, 116, 117]. By structure, coordination compounds are close to natural biological complexes responsible for the vital activity of organisms. Nature itself determined the utility of biomacromolecules for performing biological functions of vital significance that somehow explains their beneficial influence on living organisms. The catalytic action of complexes essentially differs from the action of simple ions and, similar to ferment action, is based on the creation of intermediary complexes during reaction [60].

The stimulating effect of metal complexes on the producing-microorganism enzymatic activity can be provoked by stabilizing the spatial structure of the protein molecule generating the reduction of the active center dependence on the action of external environment factors, through inclusion in the enzyme active center (in metal enzymes), or adherence to other parts of the protein globule (for example, to the enzyme allosteric center) [114].

II.2. Preliminary research for assessing the biological effect of some coordination compounds.

Currently, there is a large number of synthesized coordination compounds with different composition and structure. Their role in biology is determined by their degree of influence and effect on vital processes in humans, animals, plants and microorganisms.

Preliminary research on assessing the biological effect of a large group of coordination compounds of transition metals with organic ligands of different chemical classes – oximes, thiosemicarbazones, amino acids, proves that the introduction of coordination compounds of 3d elements in the nutrient media for cultivating fungi strains stimulates biosynthetic processes: biomass accumulation, enzyme activity increase, cultivation period reduction, culture development intensification and producent vital cycle reduction [70, 72-75, 91, 99, 119-125]. The exerted effect is influenced by the taxonomy of producer strains and the peculiarities of synthesized enzyme complexes, as well as by the structure and composition of metal complexes and the applied concentration.

Thus, the series of tested compounds, characterized by the presence of Co, Cu, Zn, Ni, V atoms – as generators of complexes, and of semicarbazide diacetic acid dehydrazide – as ligand, exerted an inhibitory action on the biosynthesis of cellulase complex, synthesized by *Penicillium expansum* CNMN FD 04 strain. The most pronounced inhibiting effect is exerted on cellobiohydrolases, while on β-glucosidases, the effect is weaker. The same coordination compounds introduced in the cultivation medium of *Aspergillus niger* 33 CNMN FD

06 strain showed a stable bio-stimulating effect on the biosynthesis of both ordinary and acid-stabile amylases [126, 127].

It has been established a differentiated influence of metal complexes on the biosynthesis of enzyme complex components synthesized by microorganisms. For example, in *Penicillium expansum* strain producing the cellulase complex composed of cellobiohydrolase, endoglucanase and β-glucosidase, the coordination compound of molybdenum with glycine $MoO_2(acac)_2Gly$ conditions only the intensification of β-glucosidases synthesis. The dioximates of Fe(II) with nicotinamide and N,N-diethylnicotinamide obviously stimulate the biosynthesis of endoglucanases and xylanases synthesized by *Aspergillus niger* USA MV strain and act as inhibitors of β-glucosidases synthesis [128-130].

It has been found that the compounds with analogous structure differently influence synthesis and enzyme activity, acting as stimulators for certain enzymatic systems, and inhibitors – for others, depending on producer strain origin (taxon) and type of synthesized enzymes.

Thus, coordination compounds of zinc with amino acids $Zn(L\text{-}Ser)_2$ and $Zn(DL\text{-}Ala)_2$ act as stimulators of *Rhizopus arrhizus* pectolytic activity, whereas in experiments carried out with the strain of *Aspergillus niger* 33 – amylase producer, the mentioned metal complexes have a neutral or inhibitory influence, generating the reduction of amylase activity by about 10-86% as compared to control [74, 131].

Th results of certain research prove that the maximum exerted effect is ensured by the integral complex structure and not by one of the constituting parts [90, 103, 132-134].

The investigation of producer strains cultivation conditions has demonstrated that the presence of metal complexes in the nutrient medium modifies the phases of culture cultivation, accelerates the earlier manifestation of enzyme biosynthesis maximum, reduces the microbial life cycle [91, 98, 135], stabilizes enzymogenesis processes under stress conditions, hence representing very important indices for creating progressive biotechnologies of fungi application in fermentation industry [73, 91].

II.3. Coordination compounds of cobalt, copper and zinc, and the perspective of their use within fungi cultivation technologies as a strategy for increasing biotechnological performances.

Among coordination compounds of preliminary tested transition metals, the complexes of cobalt(III), copper(II) and zinc(II), with ligands based on dioxime, thiosemicarbazide and amino acids, showed a broad spectrum of biostimulating activity on enzyme biosynthesis in micelial fungi.

The elements Zn, Cu, Co are vital to living organisms, including microorganisms, being involved in synthesizing lipids and proteins; in activating zymogen forms of enzymes, phosphates metabolism, and in transcripting and stabilizing the DNA molecule etc. [55, 56].

An intense bioactivity manifested the *trans*-dimethylglioximates of cobalt(III), that showed a more frequent biostimulative activity on the biosynthetic processes of the studied micromecete strains, different by gender, species and synthesized enzyme profile.

It is typical of transition metal dioximates to exhibit biological activity and, thus, be considered models of natural biological objects of vital importance, as it is, for example, vitamin B_{12} [136, 137]. It has been found that the fluorine-containing dioximates of Co(III) act as stimulators of vitamin B_{12} biosynthesis [138] and of hydrolytic enzymes in some fungi [75, 98, 103, 139-141]. Both vitamin B_{12}, and coenzyme B_{12} contain cobalt atoms [115].

The synthesis of complexes that present models of biological objects and their testing on the metabolic processes of certain microorganisms represent a perspective direction in reproducing and managing natural biosynthesis.

In this aspect, a particular interest represents the study of the influence of coordination compounds, containing as complex generating atoms the ions of the mentioned metals, on the biosynthetic ability of micromycetes producing biologically active substances, in particular, hydrolytic enzymes. Transition metal compounds with chelate ligands, including dioximates, represent complexes with a stable skeleton, various by composition, structure and performed functions [142, 143].

The synthesis of new coordination compounds with properties of favorable influence on the development of living organisms presents interest from the perspective of elaborating new efficient technologies within modern biotechnology and of proposing ergonomic solutions for the production sector. The awareness of action mechanisms, identification of active components, determination of metals and ligands role, and of their cumulative effect require a series of investigations in order to refine the methodology of oriented synthesis of biologically active substances aimed at obtaining compounds with predictable properties.This can be achieved by diversifying the nature of ligands and complex generator, which definitely influences on the variety of composition and structural architecture of assembled complexes. For investigation, there were selected mononuclear and binuclear coordination compounds of different metals (Co, Fe, Cu, Zn) with various oxidation degree (+2, +3), that include both known (MH_2, DH_2, $NioxH_2$), and newly synthesized ($DSamH_2$) mono- and bis-dioximates. The metal:dioxime ratio within complexes can be 1:1, 1:2 and 1:3.

The biological characteristics of coordination compounds were evaluated by the degree of influence on the enzymatic activity of micromycetes from different genera and species with different enzyme systems.

Strain diversification by genus, species, and profile of synthesized enzymes and enzymatic systems permits the determination of exerted effect dependence on metal complexes, fungal taxonomy position and profile of synthesized secondary metabolites.

Within investigation, there were used controlled microorganisms, preserved in optimal storage conditions: *Aspergillus niger* 33 (V. Tiegh, Rape, Fenell), CNMN FD 06 and *Aspergillus niger* 33-19 (V. Tiegh, Rape, Fenell) CNMN FD 02 – amylase producers *Rhizopus arrhizus* (Fischer 67) and *Penicillium viride* (Fresenius Beitz) CNMN FD 04 – pectinase producers, *Aspergillus niger* 412 CNMN FD 01 and *Rhizopus arrhizus* Fischer CNMN FD 03 – lipase producers, *Trichoderma koningii* Oudemans CNMN FD 15 and *Fusarium gibbosum* CNMN FD 12 – protease producers, *Aspergillus niger* (V. Tiegh) CNMN FD 10, *Aspergillus terreus*, *Aspergillus flavus* (Link), Reaper, Fenell BKM

F3292D and *Penicillium expansum* (Link) Thom CNMN FD 05 – producers of cellulases and xylanase complex. Most of enumerated strains are patented.

The cultures are preserved within the National Collection of Non-pathogenic Microorganisms created within the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova.

The research started by testing the biological effect of mononuclear complexes of Co(III) with dioximes: $[Co(DH)_2(Thio)_2]F\cdot 3H_2O$ (**1**), $[Co(DH)_2(Thio)_2][BF_4]\cdot 3H_2O$ (**2**), $[Co(DH)_2(Py)_2][BF_4]$ (**3**). These complexes are part of cobalt(III) *bis*-dioximates with *trans*-octahedral structure.

Within the complex $[Co(DH)_2(Py)_2][BF_4]$, in the equatorial plane of the central atom, coordinate two dimethylglyoxime radicals (Fig. II.1), connected through intramolecular bonds of hydrogen O-H···O with parameters O1···O4 2.514Å, O2···O3 2.481 Å, while in positions 1 and 6 of the octahedron can be found the nitrogen atoms of pyridine molecules [153]. In the external sphere can be found the anions of $[BF_4]^-$. The angle formed between the equatorial plane of the complex cation central atom and piridine molecules N5, C9, C10, C11, C12, C13 and N6, C14, C15, C16, C17, C18 constitutes 89.0 and 91.1°, respectively. Between the complex anion and cation take place electrostatic interactions F3···C12, F3···C10, F2···C5, F2···C6, equal with 3.16, 3.18, 2.92 and 2.97 Å, respectively. The presence of F2···C5 and F2···C6 contacts can be interpreted as the interaction of fluorine atom with π cloud of the metal cycle.

In order to verify the capacity to influence biosynthetic processes in fungi, the metal complexes have ben included in the cultivation medium of *Aspergillus niger* 33 CNMN FD 06 – amylase producer, used in the experiment as a biological object.

Amylases participate in the hydrolysis of starch and starch containing substrates. Amylases have a series of important advantages: their higher activity, broad specificity, presence of certain properties like termophily and acid stability, that is the capacity to resist at extremely high temperature (up to 100^o C) and low pH values (2.0-2.5). Considering the diversity of application fields, the amylases of microbial origin hold the first places among ferments. They are used in all branches of food, textile and perfume industries, in obtaining concentrated fodder, in medicine, in purifying the water, in processing residues from agriculture and industries, etc. Within theoretical research, amylases represent a favourable model for establishing the active center topography and for studying the physical and chemical properties specific of metal enzymes. Within industrial processes amylolytic enzymes are widely used for attaining glucose, syrups and various types of starch molasses. Large users of amylolytic enzymes are the alcohol and beer industry.

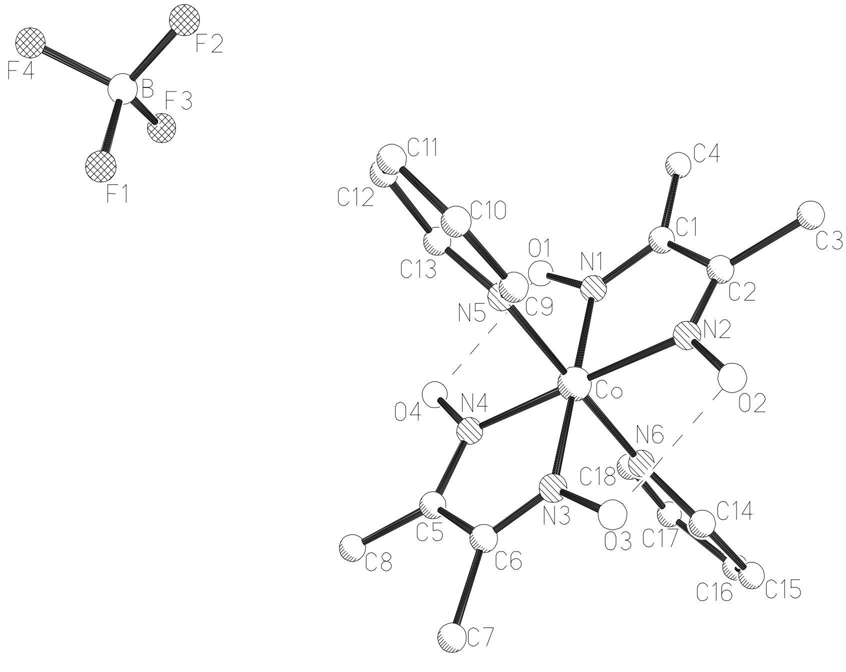

Fig. II.1. Complex structure of $[Co(DH)_2(Py)_2][BF_4]$.

The compounds were tested in three concentrations – 1, 5 and 10 mg/L. Considering the fact that the modification of the nutrient medium can influence the cultivation parameters, preliminarily established as optimal for culture cultivation in classical conditions, the investigation of new compounds effect on fungal biomass accumulation was achieved at two temperature values: 30° C (optimal) and 20° C. The obtained results are exposed in tables II.1 and II.2. The biomass was determined by weighing the dried samples. The amylolytic activity was determined in standard conditions of hydrolysis at pH 4.7 value [73].

The data exposed in table II.1 show that in favorable cultivation conditions (at 30° C), the introduction of coordination compounds slightly influenced biomass yield, the values varying within the interval 11.25-14.25 g/L, while in the control variant – 11.95 g/L or, in percentage ratio, as related to control, it constituted – 94.14-119.24%. The increase of biomass yield, even if recorded in most variants, exceeds the control variant by 5.0-19.0%.

At microorganism cultivation in conditions of low temperature (20° C) to the optimal, the supplementation with coordination compounds ensured an important increase of biomass yield: 15.25-19.10 g/L, (control – 8.20 g/L), which constitutes by 85.36-132.93% more as compared to the control sample. The optimal concentrations can be considered from 1 to 5 mg/L. The increase of coordination compounds concentration in the medium to 10 mg/L does not ensure a significant difference in biomass accumulation.

Thus, the use of complexes generates the stabilization of biosynthetic processes in unfavorable conditions of microorganism activity [73, 106]. The concentration of 5 mg/L of compounds **1**, **2**, **3** in the given experiences, can be considered optimal for both temperature values.

Table II.1.

The influence of Co(III) coordination compounds on the process of biomass accumulation in *Aspergillus niger* CNMN FD 06 in terms of temperature.

Coordination compound	Concentr., mg/L	30° C		20° C	
		Biomass, g/L CL	% compared to control	Biomass, g/L CL	% compared to control
1	1	13.75	115.06	18.70	**228.05**
	5	12.12	101.42	16.35	199.39
	10	12.75	106.69	17.05	207.93
2	1	12.62	105.60	15.75	192.07
	5	13.30	111.29	15.85	193.29
	10	11.37	95.15	17.25	**210.36**
3	1	14.25	119.24	19.10	**232.93**
	5	11.25	94.14	16.00	195.12
	10	12.50	104.60	15.27	186.28
Control	-	11.95	100	8.20	100

The study of coordination compounds influence on the amylolytic activity of *Aspergillus niger* CNMN FD 06 micromycete was carried out at fungus cultivation at the temperature of 30° C. The obtained results (Table II.2) show a beneficial influence on the biosynthesis of amylases by *Aspergillus niger* CNMN FD 06 while using compounds **1**, **2**, **3** compared to control, recording an increase of amylolytic activity in the culture liquid of the producer, by 30.73-41.65%, compared to control.

Table II.2.

The influence of complexes **1**, **2** and **3** on *Aspergillus niger* CNMN FD 06 amylolytic activity.

Coordination compound	Conc., mg/L	Amylolytic activity	
		u/mL	% compared to control
1	1	60.1	133.85
	5	61.5	137.19
	10	62.3	**138.75**
2	1	61.6	137.20
	5	63.6	**141.65**
	10	58.7	130.73
3	1	63.0	**140.31**
	5	60.1	133.85
	10	63.0	140.31
Control	-	44.9	100

Optimal concentrations vary from a compound to another, fact determined by the different composition of complexes.

The obtained results indicate that the tested dioximates stimulate biosynthetic processes in fungi (biomass accumulation, enzymatic activity increase) and contribute to their stabilization in unfavourable conditions of activity.

The second group of coordination compounds tested with the purpose to highlight the effect on the lipolytic activity of *Aspergillus niger* CNMN FD 01 strain [146], at intense cultivation, included the following complexes: $[Co(MH)_2(Py)_2][BF_4]\cdot H_2O$ (**4**), $[Co(DH)_2(An)_2][BF_4]$ (**5**), $[Co(NioxH)_2(An)_2][BF_4]$ (**6**).

Lipases have a determining role in lipid metabolism and in regulating lipid-dependent functions in living organisms. They hydrolyze esters of different origin, whose alcoholic group may be represented by glycerol (lipases), its derivatives (phospholipase) or sterines (cholesterol-esterase). Lipolytic ferments represent a unique enzyme group due to the ability to act at border phases of heterogeneous systems [118, 156, 157]. This feature increases the theoretical interest in them and broadens their practical application.

The special interest of researchers is directed towards obtaining pure enzyme preparations, explaining their action mechanisms, studying their properties (in particular re-esterification capacity, the likelihood of obtaining stereoisomers with their help) and determining the influence of different surfactants on them etc. [158-161].

The composition and structure of compounds **4-6** was studied based on natural methods of modern research. X-ray investigations showed that in $[Co(DH)_2(An)_2][BF_4]$, in the equatorial plane, there are two radicals of dimethylglyoxime, bound by intramolecular bonds of hydrogen O-H···O with the parameters O1···O3 2.528, O1-H1 0.837, O3···H1 1.697 Å and the angle O1- O1-H1···O3 170.9°. The polyhedron coordinating the cobalt atom completes to an octahedral by nitrogen atoms of aniline molecul
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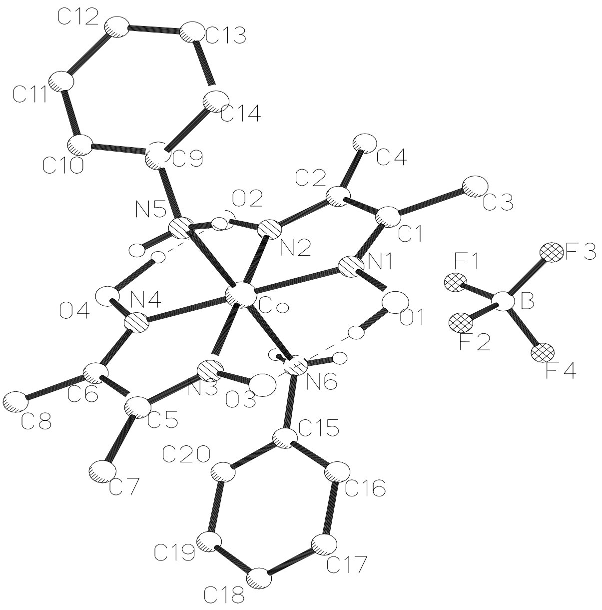


Fig. II.2. The structure of $[Co(DH)_2(An)_2][BF_4]$ molecule.

A decisive role in forming the crystal structure devolves to hydrogen bond N-H···F, between F2 atom of the complex anion and N6 of the complex cation. The parameters of hydrogen bond are: F2···N6 2.96 Å, F2···H6 2.29 Å, N6···H6 0.73 Å, the angle of hydrogen atom being of 155.5°.

All three tested complexes stimulatingly influenced the synthesis of lipases in the producer strain, the difference being marked only in the optimum concentrations ensuring the maximum effect of lipase biosynthesis: 10 mg/L for the complex with methylglyoxime (**4**), which marks the maximum value of the incentive effect – 20000 u/mL, exceeding the control by 146.15%. For the complex with dimethylglyoxime (**5**) the optimal concentration was 1 mg/L, ensuring the increase of enzyme activity by 88.24% as compared to control, and 5 mg/L for the complex with 1,2-cyclohexanedione dioxime (**6**), the increase constituting 111.76% (Tab. II.3).

Table II.3.

Modification of *Aspergillus niger* CNMN FD 01 strain lipolytic activity and biomass yield under the influence of coordination compounds.

Coordination compound	Conc., mg/L	Lipolytic activity, u/mL	% compared to control	Biomass yield, g/L	% compared to control
4	1	7.000	82.35	16.0	112.67
	5	6.000	70.59	12.0	84.50
	10	20.000	**246.15**	14.0	98.59
5	1	16.000	**188.24**	15.0	105.63
	5	9.000	105.88	17.0	119.71
	10	10.000	117.64	13.0	91.54
6	1	12.000	141.17	10.5	73.94
	5	18.000	**211.76**	15.0	105.63
	10	14.000	164.70	13.0	91.54
Control		8.500	100	14.2	100

The increase of lipolytic activity in all studied concentrations is marked only for $[Co(NioxH)_2(An)_2][BF_4]$ (**6**) complex, admitting the supposition that the complex is a specific stimulator of fungal lipases biosynthesis [162].

Biomass accumulation in these experimental variants diverged insignificantly compared to control.

The evaluation of biological properties of synthesized compounds containing sulphanilamide as a ligand: $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$ (**7**), $[Co(NioxH)_2(Sam)_2]_2[TiF_6]\cdot 3H_2O$ (**8**) and $[Co(DH)_2(Sam)_2][ZrF_6]\cdot 5H_2O$ (**9**) was achieved according to the degree of influence on the enzymatic activity of *Penicillium viride* CNMN FD 04 – pectinase producer [152] and *Aspergillus niger* 33-19 CNMN FD 02 – amylase producer [153]. The research presents interest for the biotechnology of obtaining microbial enzyme preparations aimed at increasing and stabilizing producer strains biosynthetic capacity.

Sulphanilamides are chemotherapeutic agents with a strong antibacterial activity (sulphanilic acid derivatives), very close, by chemical structure, to para-aminobenzoic acid – a necessary growth factor for microorganisms. The introduction of sulphanilamide molecules, that coordinate in the apical positions of the complex, and are known as biologically active molecules, could stimulate or inhibit certain physiological processes in cells [163].

Within the compound of $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$, two molecules of sulphanilamide are located in axial positions Lig-Co-Lig: $\nu_{as}(NH_2)_{free}=3350$, $\nu_s(NH_2)_{free}=3250$, $\nu_{as}(NH_2)_{coord}=3280$, $\nu_s(NH_2)_{coord}=3165$, $[\nu_{as}(CC)+\delta_{as}(CCH)]=1595$, $[\nu_s(CC)+\delta_s(CCH)]=1488$, $\nu_{as}(SO_2)=1312$, $\nu_s(SO_2)=1145$, $\gamma(CCC)=672$ and 562 cm^{-1}. NH_2-group is coordinated to the cobalt atom. In the external sphere of complexes can be found $[BF_4]^-$ anions, the molecules of crystallization water ($\nu(OH)_{H_2O}=3400$-3600 cm^{-1}). The bands of $[BF_4]^-$ ion are recorded within the zones of $\nu_{as}(BF_4)=1085$, $\nu_s(BF_4)=760$, $\delta(F\text{-}B\text{-}F)=525$ cm^{-1}.

In the free sulphanilamide ^1H spectrum, it manifests a singlet within 5.79 ppm region, specific of NH_2 group, directly bound to the aromatic ring, two doublets, 6.59 ppm and 7.75 ppm, belonging to the aromatic ring protons and a singlet within 6.88 ppm, characterizing NH_2 group, far from the aromatic ring. At coordination, can be observed the signals displacement of NH_2 group bound to SO_2 group by ~0.5 ppm and of those of CH groups close to SO_2 group by ~0.13 ppm. The other signals vary within the limits of 0.03–0.07 ppm.

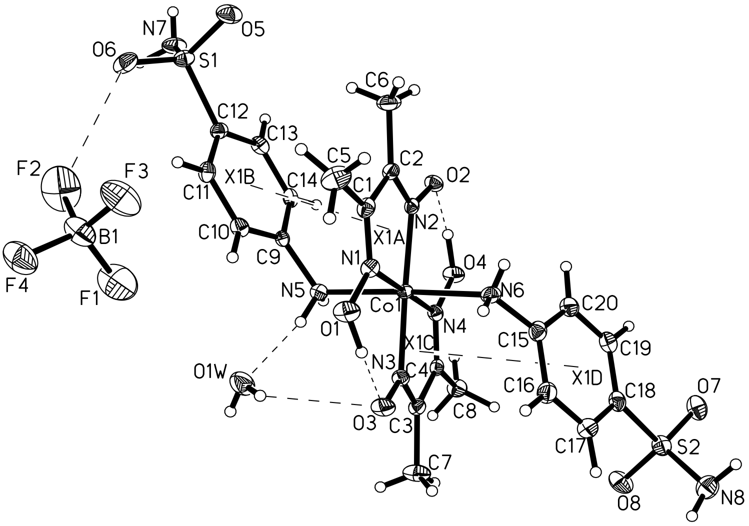
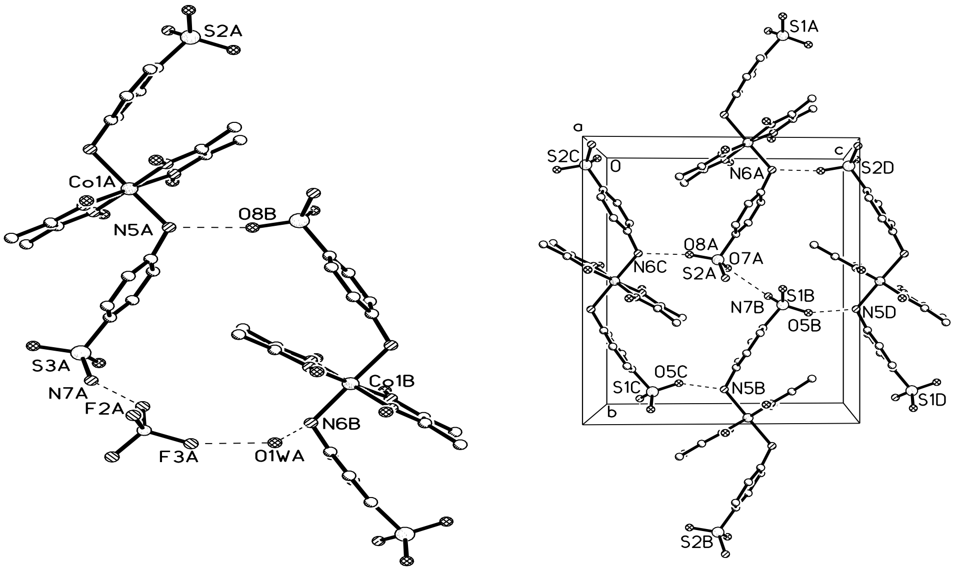


Fig. II.3. The structure of $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$ complex.

The structure of $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$ (Fig. II.3) includes $[Co(DH)_2(Sam)_2]^+$ cation, $[BF_4]^-$ anion and the molecule of crystallisation water.

Band pieces C9···C14 and C15···C20 of sulphanilamide are practically parallel to the equatorial planes of metal cycles Co1N1N2C1C2 and Co1N3N4C3C4, forming angles of 25.7(1) and 27.8(1)°, and π-π interactions with distances between centers of 3.488(2) and 3.469(2) Å [164].

Within the crystal, there accomplish cyclic fragments both with (II.4a) and without (Fig. II.4b) the participation of complex anions.



a b

Fig. II.4. Formation of cyclic fragments within $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$.

Within the $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$ complex, form the hydrogen bond N8-H···F4 [-x+2, -y, -z+1] 2.889(3) Å and the stable intermolecular contact N8-H···F2 [x, 1+y, z] 2.872(3) Å. The centrosymmetric motives are joined between them through O4···O4 [1-x, -y, 1-z] 2.717(3) Å intermolecular contacts and N7-H7A···O7 [x, y-1, z] 2.878(3) Å hydrogen bond.

Data obtained in biological research highlight the differentiated effect of Co(III) dioximates with sulphanilamide on the biosynthesis of pectolytic enzyme complex components synthesized by *Penicillium viride* CNMC FD 04P strain [145] and amylolytic of *Aspergillus niger* 33-19 CNMN FD 02A strain [144].

The results exposed in table II.4 show a clear stimulation of pectinase activity under the action of $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$ compound. The enzymatic activity is higher, as compared to control, both on day 3, and day 4 of producer cultivation, constituting 450.28-498.37 u/mL and 842.31-860.71 u/mL in experimental variants, depending on the applied concentration and cultivation term, compared to 437.39-611.30 u/mL in the control variant.

The complex does not affect the developmental stages of the organism. Maximum synthesis of pectinases in experimental variants are recorded on day 4 of cultivation and coincides with the maximum of the control variant. The optimum concentration of the complex constitutes 5 to 10 mg/L and ensures the increase of pectolytic activity by 37.8-40.8% as compared to control.

Table II.4.

Modification of *Penicillium viride* CNMN FD 04 P micromycete pectolytic activity (PA) under the influence of compounds **7** and **8**.

Coordination compound	Conc., mg/L	On the 3-rd day of cultivation		On the 4-th day of cultivation	
		PA, u/mL	% compared to control	PA, u/mL	% compared to control
7	1	498.37	113.9	526.89	86.2
	5	463.74	106.0	860.71	140.8
	10	450.28	102.9	842.31	137.8
8	1	457.26	104.5	658.27	107.7
	5	451.48	103.2	724.07	118.4
	10	339.04	77.5	557.64	91.2
Control	0	437.39	100	611.30	100

The metal complex of $[Co(NioxH)_2(Sam)_2]_2[TiF_6]\cdot 3H_2O$ in the variants with smaller concentrations (1.0-5.0 mg/L) practically doesn't influence the strain enzymatic activity, exerting an inhibiting action up to 12.3-26.0% at a more increased concentration – 10 mg/L. The different influence of coordination compounds on the micromycete pectolytic activity can be conditioned by the different composition of metal complexes. Complexes contain not only different anions $[BF_4]^-$, $[TiF_6]^{2-}$, they also differ by equatorial ligands structure represented by DH and NioxH – monoanions of dimethylglyoxime and respectively of 1,2-cyclohexanedione dioxime [165].

Despite the results obtained for *Penicillium viride* strain, in the experiments on *Aspergillus niger* 33-19 strain, the compound $[Co(NioxH)_2(Sam)_2]_2[TiF_6]\cdot 3H_2O$ manifests as a real stimulator of amylase biosynthesis.

In all tested concentrations, it was recorded an increased activity of both types of amylases – acid-labile and acid-stable, respectively by 40.47-164.08% and 33.9-148.15% (Table II.5).

Table II.5.

Modification of acid-labile amylase activity in *Aspergillus niger* 33-19 CNMN FD 02 micromycete under the influence of compounds **8** and **9**.

Complex	Conc., mg/L	On the 5-th cultivation* day		On the 6-th cultivation* day	
		activity u/mL	% compared to control	activity, u/mL	% compared to control
8	1	286.26±13.0	141.54	238.23±3.60	117.79
	5	331.85±6.51	**164.08**	250.93±3.64	124.07
	10	284.09±3.76	140.47	227.65±3.35	112.56
9	1	173.81±2.12	95.8	87.46±2.32	47.1
	5	157.77±1.34	87.0	74.97±1.98	40.4
	10	117.64±2.64	64.9	64.13±2.09	34.6
Control	0	172.63±5.58	100	202.25±0.28	100

*enzymatic activity in variants is exposed in relation to the control variant maximum value.

The complex modifies the stages of strain development, expressed by the acceleration of enzymes biosynthesis maximum manifestation by 24 hours as compared to control (Table II.5, II.6).

As for the effect exerted by $[Co(DH)_2(Sam)_2]_2[ZrF_6]\cdot 5H_2O$ compound, with zirconium anion $[ZrF_6]^{2-}$, on the enzymatic activity of *Aspergillus niger* 33-19 strain, it diminishes amylase activity, even if culture development keeps the trend of the compound variant with titanium anion $[TiF_6]^{2-}$ – the earlier manifestation of biosynthesis maximum (on day 5) as compared to control, yet amylase activity remains under the level of control variant 173.81-157.77 u/mL as compared to 202.25 and 248.78-244.01 u/mL to 280.56 u/mL, for acid-labile and acid-stable amylases.

Table II.6.

Modification of acid-stable amylase activity in *Aspergillus niger* 33-19 CNMN FD 02 micromycete under the influence of compounds **8** and **9**.

Complex	Conc., mg/L	On the 5-th cultivation*day		On the 6-th cultivation*day	
		activity u/mL	% compared to control	activity, u/mL	% compared to control
8	1	375.75±4.61	133.90	269.98±3.67	96.23
	5	415.65±4.71	**148.15**	316.55±0.26	112.80
	10	383.27±4.09	136.60	255.17±3.43	90.95
9	1	248.78±2.18	88.67	209.80±2.32	74.78
	5	244.01±1.64	87.00	235.97±1.98	84.11
	10	182.08±2.53	64.9	217.23±2.09	77.42
Control	0	262.38±3.76	100	280.56±3.61	100

* enzymatic activity in variants is exposed in relation to the control variant maximum value.

It has been found that the effect of coordination compound varies significantly both in terms of compound composition/concentration, strain origin and profile of the synthesized enzymatic complex.

According to research results, $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$ and $[Co(NioxH)_2(Sam)_2]_2[TiF_6]\cdot 3H_2O$ coordination compounds can be recommended for the development of new proceedings of increased oriented synthesis of pectinase and amylase in biosynthesis in mycelial fungi.

The study of $[Co(DH)_2(An)_2][PF_6]$ complex (**10**), under the structural aspect, allowed the elucidation of some properties offered by the nature of ligands and the complex anion. The presence of $[PF_6]^-$ anion in the external sphere is proved by the signal within NMR ^{19}F to -69.4 ppm ($\delta[PF_6]^-$) spectrum. Complex cations of $[Co(DH)_2(An)_2]^+$, by means of amine groups of coordinated An molecules, participate in the formation of intermolecular hydrogen bonds N(3)–H···O(2)($-x$+1, $-y$+2, $-z$), (donor···acceptor 3.019(3) Å), that allow the formation of the unidimensional chain oriented along the crystal *y* axis [164].

Cation chains are additionally stabilized by means of C(21)–H···O(1)(*x*, *y*+1, *z*) intermolecular hydrogen bonds, (donor···acceptor 3.313(3) Å, C(21)–H 0.96, H···O(1) 2.40 Å, angle CHO 159°), while by means of N–H···F (donor···acceptor 3.063(4) Å) intermolecular hydrogen bonds, they are connected in layers along the crystal *z* axis.

Given the system of $Co(CH_3COO)_2·4H_2O$ - KPF_6 - $2NioxH_2$ - 2Thio in the medium $H_2O:CH_3OH$:DMF (3:1:1), it was obtained the complex of $[Co(NioxH)_2(Thio)_2][PF_6]·0.5DMF·0.5H_2O$ (**11**), in whose IR spectrum, the absorption bands ν(CN)=1562, ν_{as}(NO)=1220, ν_s(NO)=1060, γ(OH)=980, γ(CNO)=725, ν_{as}(Co-N)=535 and ν_s(Co-N)=430 cm^{-1} can be attributed to 1,2-cyclohexanedione dioxime monoanion coordinated to the metal ion, while ν_{as}(NH)=3312, ν_s(NH)=3210, $\delta(NH_2)$=1615, [ν(CN)+ν(CS)+δ(HNC)]=1060, δ(NCS)=412 cm^{-1} oscillations indicate on the presence of thiocarbamide molecules in the complex.

Alternating, **A** and **B** $[Co(NioxH)_2(Thio)_2]^+$ complex cations form, by means of N(4A)–H···O(2B)(–*x*+1, –*y*+1, –*z*+1) (N···O 2.870(8) Å) and N(3B)–H···O(1A)(*x*, *y*, *z*) (N···O 2.870(9) Å) intermolecular hydrogen bonds, chains oriented along the crystal *z* axis (Fig. II.5) [164].

The chains connect in layers on the basis of N(4B)–H···F(1)(–*x*+1, –*y*, –*z*+1) (N···F 3.03(2) Å), N(4B)–H···F(1)(*x*, *y*, *z*) (N···F 3.15(3) Å), N(4B)–H···F(6)(*x*, *y*, *z*) (N···F 3.11(3) Å) and C(14)–H(1)···F(4)/F(2A)/F(6B)(–*x*+1, –*y*, –*z*+1), (donor···acceptor 3.382(6)/3.409(5)/3.345(5) Å, C(14)–H(1) 0.97, H···F(4)/F(2A)/F(6B) 2.53/2.60/2.43 Å, angle CHF 146/141/157°) intermolecular hydrogen bonds, where fluorine atoms of $[PF_6]^-$ anions are involved as acceptors.

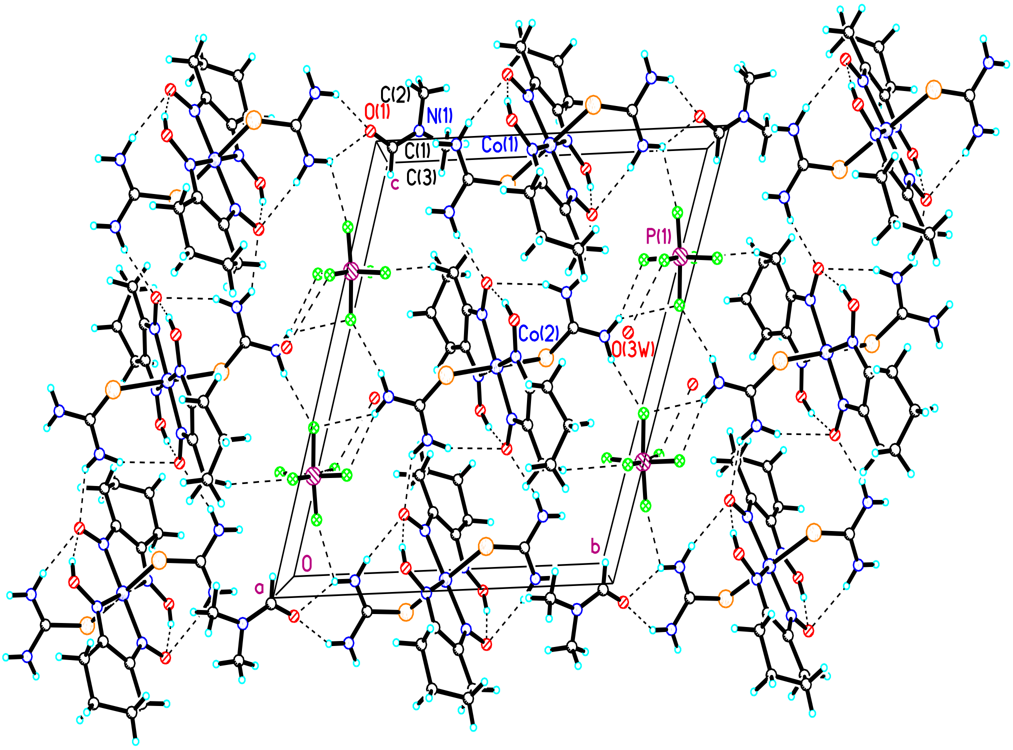


Fig. II.5. Fragment of $[Co(NioxH)_2(Thio)_2][PF_6]\cdot0.5DMF\cdot0.5H_2O$ casing in crystal.

Within the gaps of the crystalline net can be found dimethylformamide molecules, connected by intermolecular hydrogen bonds to the chains of N(3A)–H···O(1)(*x*, *y*+1, *z*) (N···O 2.850(10) Å) and N(4A)–H···O(1)(*x*, *y*+1, *z*) (N···O 2.922(10) Å) complexes, where oxygen atoms and irregular water molecules, bound by different weak intermolecular hydrogen bonds: O(W)–H···F (O···F 3.108(10)–3.294(10 Å), act as acceptors.

The biological properties of $[Co(DH)_2(An)_2][PF_6]$ (**10**) and $[Co(NioxH)_2(Thio)_2][PF_6]\cdot0.5DMF\cdot0.5H_2O$ complexes (**11**) were evaluated according to the degree of influence on the process of synthesizing acid and neutral proteases in *Fusarium gibbosum* CNMN FD 12 micromycete [139].

Proteases are the single class of enzymes, which occupy a pivotal position with respect to their applications in both physiological and commercial fields. Proteases are degradative enzymes that catalyze the total hydrolysis of proteins. Advances in analytical techniques have demonstrated that proteases conduct highly specific and selective modifications of proteins such as activation of zymogenic forms of enzymes by limited proteolysis, blood clotting and lysis of fibrin clots, and processing and transport of secretory proteins across the membranes. They are responsible for the complex processes involved in the normal physiology of the cell, as well as in abnormal pathophysiological conditions. The vast diversity of proteases, in contrast to the specificity of their action, has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications [166-168]. Taking into account the broad application of proteases in a series of industries (food, textile, production of detergents), medicine and pharmacology, the study of the influence of cobalt coordination compounds with dioximes on the biosynthesis of exocellular proteases in *Fusarium gibbosum* micromycete presents a scientific and practical interest.

Investigation results have confirmed the stimulating effect of the tested coordination compounds on the activity of acid and neutral proteases in *Fusarium gibbosum* CNMN FD 12 micromycete. The effect value varies depending on the applied concentration (Table II.6; Fig. II.6**)**. In all samples the maximum activity was recorded on the 5-th cultivation day, concurrently with the manifestation of activity maximum in the control sample.

The complex $[Co(DH)_2(An)_2][PF_6]$ (**10**) increases the activity of acid proteases by 12.1-48.5% (3.108-4.116 compared to 2.772 u/mL in the control variant, reaching the maximum value at the concentration of 10 mg/L. In the case of neutral proteases, activity increased by 17.5-92.5%, the maximum level being ensured by the same concentration (10 mg/L). On the 4-th day of cultivation, in the presence of the coordination compound in the concentration of 5 mg/L, the activity of neutral proteases was by 10% higher than the maximum activity reached in the control sample on the 5-th day (the day of maximum manifestation in conditions of control), fact showing on the increase of cell capacity to synthesize the enzyme. At cultivating micromycetes on a medium containing complex **11**, the activity of acid proteases exceeds the activity of control variant by 15.2-63.6%, the maximum activity being reached at the concentration of 5 mg/L.

As for the neutral protease activity, the experimental variant is by 50.0-87.5% higher as compared to control, being achieved at complex concentration of 5 mg/L.

Alike $[Co(DH)_2(An)_2][PF_6]$ maximum, the coordination compound of $[Co(NioxH)_2(Thio)_2][PF_6]\cdot 0.5DMF\cdot 0.5H_2O$ enhances protease biosynthesis within the cell.

On the fourth day, the activity of acid and neutral protease, at some concentrations, is practically equivalent to control on the fifth day. The appearance of biosynthesis maximum 24 hours earlier in experimental variants testifies to the intensification of all phases of organism development, thus shortening the technological process [170].

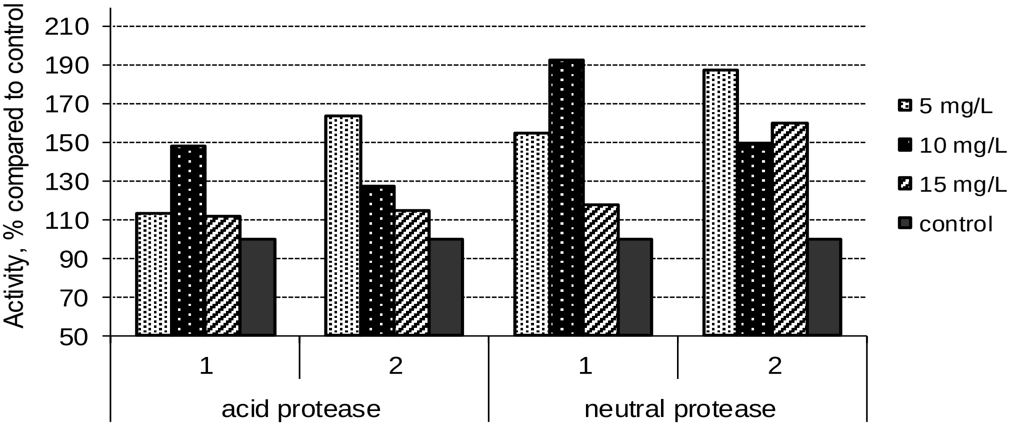


Fig. II.6. The influence of complexes **10** – $[Co(DH)_2(An)_2][PF_6]$ and **11** – $[Co(NioxH)_2(Thio)_2][PF_6]\cdot 0.5DMF\cdot 0.5H_2O$) on acid and neutral protease activity of *Fusarium gibbosum* (on the 5-th day of cultivation).

Next, it was made an attempt to produce a new class of coordination compounds – complexes of cobalt(III) with dimethylglyoxime, and namely fluoride-hexafluorophosphate-*bis*-[di(thiocarbamide)bis(dimethylglyoximato) cobalt(III)], which can be used as biostimulator and catalyst in various chemical and biotechnological processes.

Table II.6.

Modification of *Fusarium gibbosum* CNMN FD 12 proteolytic activity under the influence of $[Co(DH)_2(An)_2][PF_6]$ (**10**) and $[Co(NioxH)_2(Thio)_2][PF_6]\cdot 0.5DMF\cdot 0.5H_2O$ (**11**) coordination compounds.

Coordination compound	Conc., mg/L	Activity of acid proteases, u/mL			Activity of neutral proteases, u/mL		
		Day 4	Day 5	Day 6	Day 4	Day 5	Day 6
10	5	0.336±0.01	3.15±0.04	0.420±0.04	**3.696 ±0.07**	5.208±0.07	2.352±0.04
	10	0.42±0.04	**4.116±0.04**	0.504±0.04	2.772 ±0.04	**6.468±0.04**	0.504±0.05
	15	0.756±0.07	3.108±0.04	0.392±0.02	2.856 ±0.07	3.948 ±0.07	0.392± 0.02
11	5	0.756±0.04	**4.53±0.03**	1.596±0.04	**3.612 ±0.04**	**6.30 ±0.04**	2.52±0.07
	10	1.512±0.01	3.528±0.04	1.512±0.04	3.024 ±0.07	5.04 ±0.08	3.864 ±0.04
	15	**2.688±0.04**	3.192±0.07	0.420±0.04	3.108 ±0.01	5.376 ±0.08	1.932 ±0.07
Control	-	0.504±0.04	2.772±0.04	1.176±0.07	1.26 ±0.01	3.36 ±0.07	2.52 ±0.04

The compound $[Co(DH)_2(Thio)_2]_2F[PF_6]$ **(12)** completes the series of dioximates with anions that contain the fluorine atom. In this structure it has been discovered a totally unusual way of casing structural units, an arrangement with a very high cubic symmetry. Cations form a network, whose cavities are occupied by anions F^- and $[PF_6]^-$. From this point of view, it can be attributed to classical inclusion compounds of „guest-host” type, where the „host” role is fulfilled by urea or thiourea molecules, forming cavities in the shape of hexagonal tunnels. This complex is the first of this class of substances for which, following a review with X-ray, it was established the inclusion of $[PF_6]^-$ anion. Additionally, the structure of the given compound includes two anions of a different nature – F^- and $[PF_6]^-$ [171].

For Co(III), from the independent symmetriccation of the elementary cell fundamental domain, can be obtained the coordination polyhedron of octahedral shape. Due to the central metal ion location in a particular position, for coordinated ligands, it was independently established, from the symmetrical point of view, by a half of those two dimethylglioxime monoanions and a thiocarbamide molecule, arranged in general position.

As a result, the coordination polyhedron of Co(III) is carried out by four nitrogen atoms of the two bidentate ligands of dimethylglyoxime and two sulfur atoms that belong to the two monodentate molecules of thiocarbamide. Following dimethylglyoxime coordination, there form two basically coplanar (the dihedral angle between the planes passing through metal cycles atoms is equal to 1.8°) metal cycles. We highlight that the latter are located in the equatorial plane of the complex cation, while in the axial position can be found thiocarbamide molecules, fact showing that we have obtained results that are consistent with those previously established for compounds with similar structural peculiarities. For the complexes of this type, it is very particular the reciprocal arrangement of equatorial and axial ligands, as square plane position of chelates is additionally cemented in plane by two intramolecular hydrogen bonds O-H···O (O···O distance is equal to 2.559 Å). As stated previously, thiocarbamide molecules can be positioned both perpendicularly to the equatorial plane of the complex cation and virtually parallelly, while the intramolecular interactions of these units will be different: in the first case we'll get hydrogen bonds, which will link some of the two amino groups of thiocarbamide molecules with oxime oxygen atoms; and in the second case, there will be π-π type interactions between thiocarbamide molecules and metal cycles. Figure II.7 shows that within the given complex cation it is performed the first case, the dihedral angle between the planes formed by four coordinate nitrogen atoms and the fragment practically plane of thiocarbamide equals 120.9°. In those two similar hydrogen bonds, fact determined by cation symmetry, N···O distance is equal to 2.934 Å.

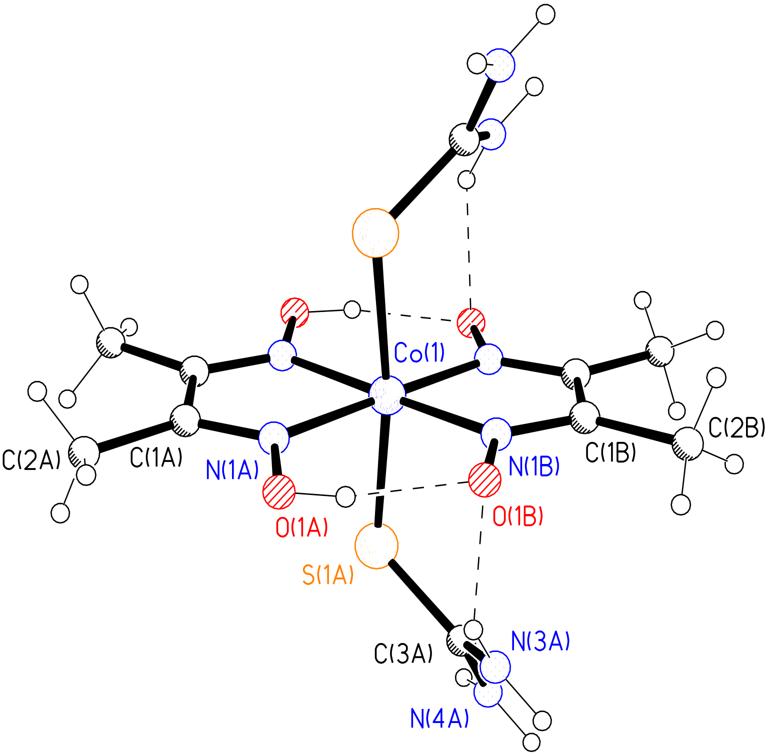


Fig. II.7. Structure of the complex cation, determined by cobalt atom particular position.

Within the complex of $[Co(DH)_2(Thio)_2]_2F[PF_6]$ as proton donors act amine groups of thiocarbamide molecules from the complex cation composition, while as acceptors act both fluorine ions and fluorine atoms from the composition of $[PF_6]^-$ anion, and the oxygen atoms of oxime groups.

Figure II.8 shows that in cation network formation, an important role play the two hydrogen bridges of N-H···O type between amine groups of thiocarbamide coordinated molecules and oxime groups of adjacent complex compounds.

The parameters of this hydrogen bridge are as follows: N···O = 2.952 Å, N-H = 0.900 Å, O···H = 2.161 Å, ∠NHO = 146.2°; N···O = 2.942 Å, N-H = 0.900 Å, O···H = 2.130 Å, ∠NHO = 149.6°.

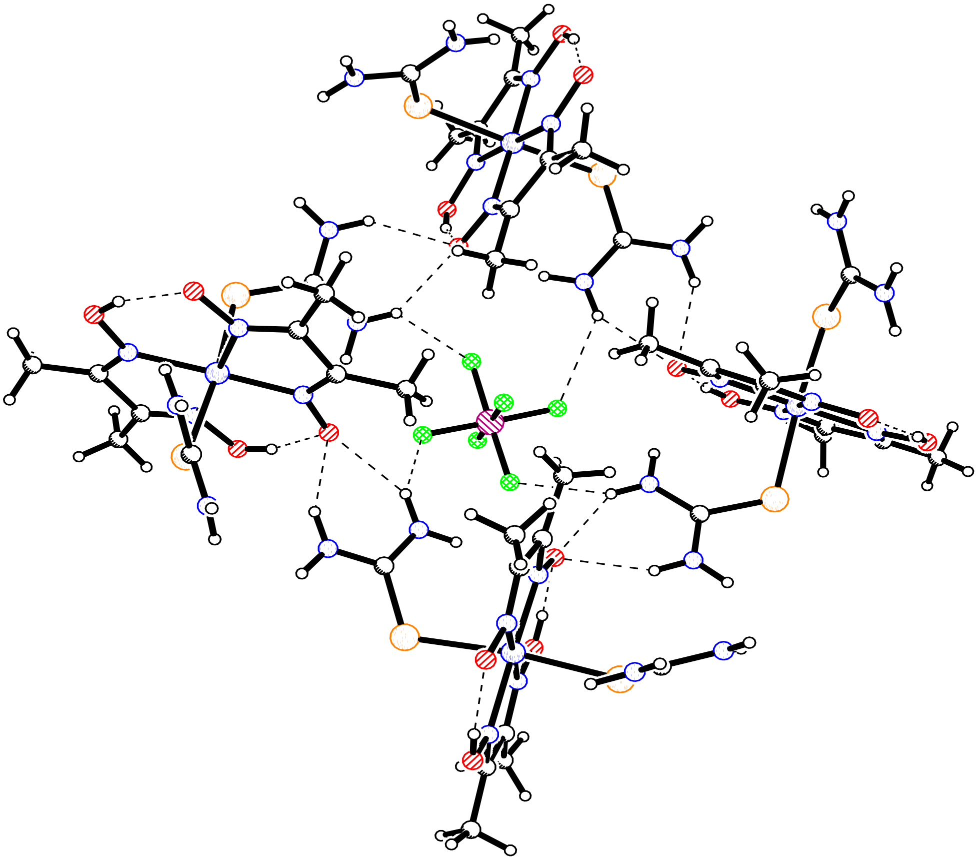


Fig. II.8. Four complex cations form tunnels occupied by $[PF_6]^-$ anions.

Each $[PF_6]^-$ anion, throughout fluorine atoms, is involved in the formation of hydrogen bridges of N-H···F type. All the more: each hexafluorophosphate anion is bound through these hydrogen bridges with four cations, and in the formation of the bridge system surrounding the anion participate only the fluorine atom located in general position F(1), while the four mentioned complex cations are symmetrical through the symmetry axis of order four.

The parameters of this hydrogen bridge are as follows: N···F = 3.145 Å, N-H = 0.900 Å, F···H = 2.485 Å, ∠NHF = 130.6°. As for the other two fluorine atoms located in particular position, one of them, namely F(2), is involved in the formation of four weak bonds of hydrogen with C-H proton donor groups of methyl groups, and the other – F(3) – participates in a weak interaction of F···F type with the interatomic distance of 2.634 Å.

The obtained results emphasize both the unusual combination of intermolecular non valent interactions, due to the different mutual arrangement of ligands in the complex cation of $[Co(DH)_2(Thio)_2]_2F[PF_6]$, and the existence of those two different by nature anions.

It is important to note that the fluorine ion is statistically located in two positions. F^- ion, placed in the center of symmetry, binds through hydrogen bridges with six complex cations symmetrically bound among themselves through the inversion axis of order three. The parameters of this hydrogen bridge are N···F = 2.816 Å, N–H = 0.900 Å, F···H = 1.926 Å, ∠NHF = 169.7°.

As a result of using $[Co(DH)_2(Thio)_2]_2F[PF_6]$ complex (**12**) in the nutrient medium of *Fusarium gibbosum* CNMN FD 12 fungal strain, It has been found the reduction of cultivation period by 24 hours, the increase of neutral proteases biosynthesis by 56.36-58.92% as compared to the prototype, maintaining the level of activity of other components of the enzyme complex (acid proteases, xylanase, β-glucozidaze) synthesized by the producer strain [169].

The activity of micromycete neutral proteases (pH 7.4) determined in the cultural liquid by Anson method, after action on sodium caseinate, had been recorded, on the fifth cultivation day, ranging between 45.02-53.13 u/mL, depending on the applied concentration of the coordination compound, with maximum manifestation at the concentration of 0.0015%, as compared to 33.43 u/mL (on the 6-th cultivation day – the day of maximum manifestation of strain biosynthesis on prototype medium), in the control variant, the increase of activity constituting 58.92% (Table II.7).

The activity of β-glucosidases and xylanases, determined by dozing reducing sugars amount, released under the influence on specific substrates-n-nitrophenyl β-D-glucopyranoside and birch xylan, constituted 2.29 and 6.10 u/mL respectively, showing the level of control variant.

Table II.7.

Modification of neutral protease activity during the cultivation of *Fusarium gibbosum* CNMN FD FD 12 in the presence of $[Co(DH)_2(Thio)_2]F[PF_6]$ coordination compound.

Coord. comp.	Conc. CC, mg/L	Proteolytic activity			
		On the 5-th day		On the 6-th day	
		u/mL	% compared to control on the 6-th day	u/mL	% compared to control on the 6-th day
12	5	46.34	138.62	31.61	94.55
	10	47.67	142.59	39.55	118.31
	15	53.13	**158.92**	40.72	121.80
	20	45.02	134.67	37.07	110.90
Control	-	25.10	100	33.43	100

(Calculations are made in relation to biosynthesis maximum of the control medium – the 6-th cultivation day)

Out of the $CoSiF_6 \cdot 6H_2O – 2DH_2 – 2Thio$ system, it was obtained the $[Co(DH)_2(Thio)_2]_2[SiF_6] \cdot 3H_2O$ complex, and at supplementation with hydrofluoric acid, it was obtained the $[Co(DH)_2(Thio)_2]_3F[SiF_6] \cdot 1.5H_2O$ complex, whose composition and structure had been studied [70, 71, 140, 141, 171]. The biological properties of newly synthesized cobalt(III) dioximates, containing hexafluorosilicate anions: $[Co(DH)_2(Thio)_2]_3F[SiF_6] \cdot 1.5H_2O$ (**13**) and $[Co(DH)_2(Thio)_2]_2[SiF_6] \cdot 3H_2O$ (**14**), were determined through testing on enzymatic activity and productivity of micromycete strains: *Aspergillus niger* CNMN FD 06 – amylase producer, *Aspergillus niger* CMNM FD 01 – lipolytic enzyme producer [146] and *Aspergillus flavus* VKM F 3292 D – cellulase producer [152]. The compounds were introduced in basic media for cultivation in concentrations of 1-5 mg/L.

The research results showed an increase of biomass productivity in all three cultures, the growth representing 52.76, 36.13 and 54.35%, as compared to control (Table II.8) [70, 71, 140, 141, 164, 165].

Table II.8.

The influence of $[Co(DH)_2(Thio)_2]_3F[SiF_6]\cdot1.5H_2O$ (**13**) and $[Co(DH)_2(Thio)_2]_2[SiF_6]\cdot3H_2O$ (**14**) complexes on biomass accumulation in *Aspergillus* genus fungi.

Comp.	Concentr., mg/L	*A. niger CNMN FD 06*			*A. niger CNMN FD 01*			*A. flavus VKM F 3292 D*		
		biomass			biomass			biomass		
		g/L	% compared to control	%	g/L	% compared to control	%	g/L	% compared to control	%
13	1	3.04	152.76	**52.76**	2.11	136.13	**36.13**			
	5	2.47	124.12	24.12	1.86	120.00	20.00	1.42	154.35	**54.35**
	10	2.49	125.12	25.12	1.79	115.48	15.48	1.25	135.87	35.87
	15	-	-	-	-	-	-	1.13	122.82	22.82
14	1	2.65	133.16	33.16	1.81	116.77	16.77	-	-	-
	5	2.87	144.22	**44.22**	2.18	140.64	**40.64**	1.03	111.95	17.95
	10	2.70	135.67	35.67	1.69	109.03	9.03	1.15	125.00	**25.00**
	15	-	-	-	-	-	-	1.09	118.47	18.47
Control	-	1.99	100	-	1.55	100	-	0.92	100	-

For compound **13**, the accumulation of biomass in *A. niger* CMNM FD 06 and *A. niger* CMNM FD 01 strains, in big amounts, was remarked at the concentration of 1 mg/L, and for $[Co(DH)_2(Thio)_2]_2[SiF_6]\cdot 3H_2O$ the maximum of biomass accumulation was recorded at the concentration of 5 mg/L. The highest rate of biomass accumulation for *A. flavus* VKM F 3292 D was reached at concentrations of 5 and 10 mg/L of both **13** and **14** compounds included in the nutrient medium.

As biological objects, to test compounds **13** and **14** as stimulators of extracellular hydrolase biosynthesis, served the strains of *Rhizopus arrhizus* Fischer 67 – producer of enzymes with pectolytic activity and *Aspergillus niger* CNMN FD 06 – amylolytic enzyme producer.

Both compounds exhibit superior stimulating effect on pectinase biosynthesis in *Rhizopus arrhizus* strain. The increase of pectolytic activity represents 29.52-184.08% as compared to the control variant, depending on the applied concentration of dioximates and cultivation period duration – 48 and 72 hours (Table II.9).

In the experimental variants, the maximum biosynthesis for both complexes manifests within 48 hours (on the 2-nd day) of cultivation and constitutes 91.50 u/mL and 99.30 u/mL at the concentration of 5 mg/L, 92.80 u/mL and 99.43 u/mL at the concentration of 10 mg/L, for **13** and **14** respectively, as compared to 35.0 u/mL in the control variant, exceeding it by 162.57% and 183.71% and 165.14% and 184.08% respectively at concentrations of 5 and 10 mg/L. With a more decreased difference, enzyme synthesis in the experimental variants, keep superior to control, even after 72 cultivation hours – the term of maximum biosynthesis in the control variant (40.14 u/mL).

In percentage ratio, the increase, as compared to the control variant, constitutes 97.15 and 87.46% at the concentration of 5 mg/L; 115.35 and 135.50% at the concentration of 10 mg/L, for compounds **13** and **14**, their effectiveness being close, fact conditioned by their similar structures [106, 174].

Table II.9.

The influence of compounds **13** and **14** on the pectolytic activity of *Rhizopus arrhizus* at cultivation within 72 and 46 hours.

Coordination compound	Compound concentration, mg/L	Cultivation within 72 hours		Cultivation within 48 hours	
		Pectinase activity, u/mL	% compared to control	Pectinase activity, u/mL	% compared to control
13	1	51.99±0.36	129.52	58.03±0.41	165.80
	5	79.14±0.48	197.15	91.50±0.55	262.57
	10	86.44±0.27	**215.35**	92.80±0.23	**265.14**
14	1	52.13±0.62	129.87	55.70±0.34	159.14
	5	75.25±0.51	187.46	99.30±0.71	283.71
	10	94.53±0.33	**235.50**	99.43±0.45	**284.08**
Control	-	40.14	100	35.00±0.52	100

Therefore, the presence of coordination compounds investigated in the nutrient medium increases the final product yield and extends the stationary phase by 24 hours, this being a positive index for a producer with biotechnological significance. Amylase biosynthesis is slightly influenced by the presence of cobalt(III) dioximates in the medium. To enhance the biosynthetic capacity of fungal strains, there were used new cobalt(III) dioximates, containing organic molecules in the internal sphere (dimethylglyoxime, 1,2-cyclohexanediondioxime, aniline, nicotinamide), and fluorinated anions in the external sphere $[ZrF_6]^{2-}$, $[BF_4]^-$, $[TiF_6]^2$: $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$ (**15**), $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$ (**16**), $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$ (**17**), $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ (**18**) [164, 173].

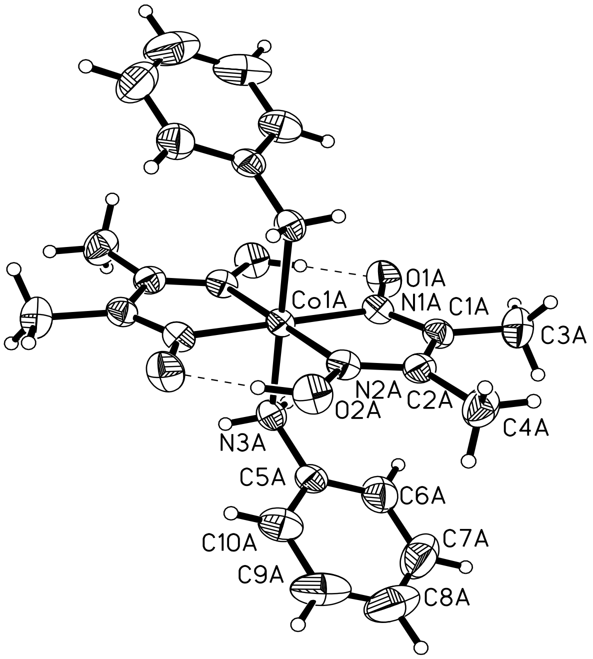


Fig. II.9. The structure of complex cation **A** in $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$.

The structure of $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$ compound represents a supramolecular complex formed of two crystalographically independent centrosymmetric cations $[Co(DH)_2(An)_2]^+$ **A** (Fig. II.9) and **B,** connected through hydrogen bonds, formed of crystallization water and $[ZrF_6]^{2-}$ complex anion. Dimethylglyoxime residues are arranged in a plane, while aniline molecules occupy the apical positions of the octahedron [164]. Similar to the case of complexes containing thiocarbamide, in the formation of coordination octahedron participate 4 nitrogen atoms that belong to the two radicals of dimethylglyoxime and two aniline nitrogen atoms which complete the coordination number of cobalt to 6. The average distances $(Co\text{-}N)_{DH^-}$ and $(Co\text{-}N)_{An}$ in **A** and **B** are different and constitute 1.895(2) and 2.009(2) Å respectively. Among oxime groups of dimethylglyoxime residues there are intramolecular hydrogen bonds O1B-H1B···O2B and O2A-H2A···O1A, equal to 2.500 and 2.486 Å correspondingly, with hexaatomic pseudocyles formation. The dihedral angles formed between the aromatic rings of aniline molecules C5A-C10A and C5B-C10B with pentatomic metal cycles Co1A, N1A, N2A, C1A, C2A and Co1B, N1B, N2B, C1B, C2B make 31.6(1) and 28.7(1)° respectively. The distance between aromatic rings centers and metal cycles is equal to 3.81(2) and 3.64(2) Å, fact admitting the possibility of some weak π-π interactions (Fig. II.10).

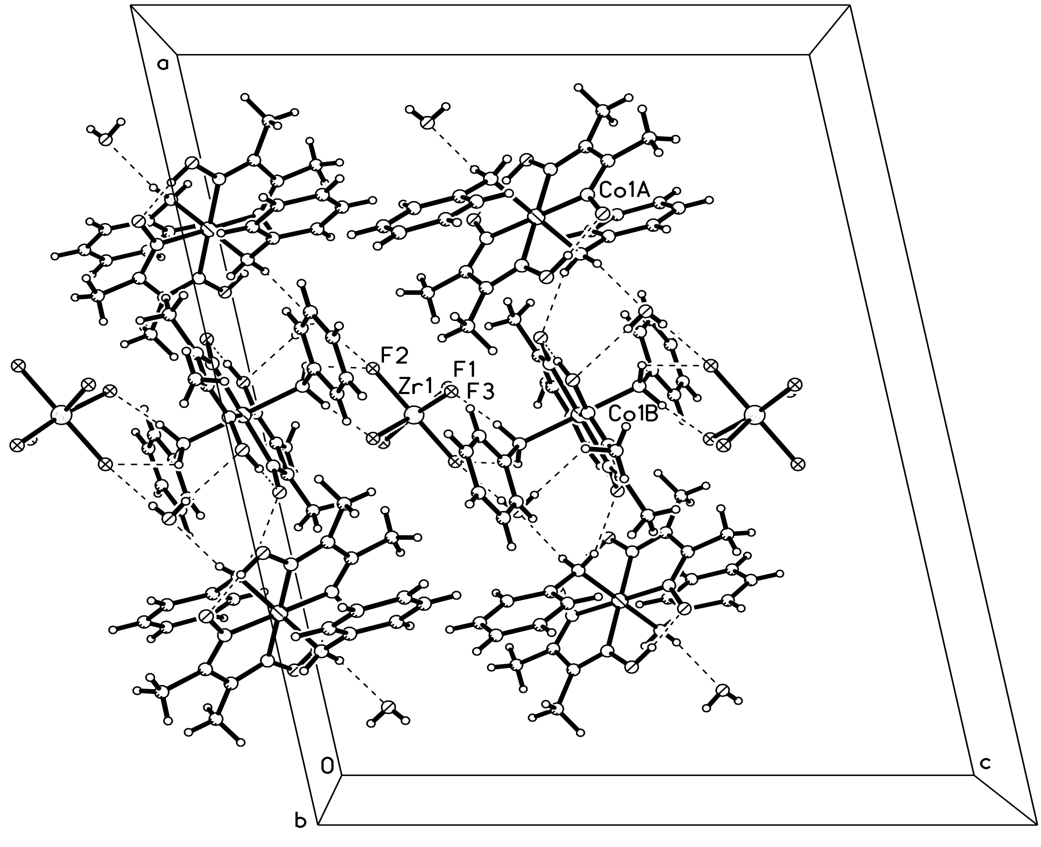


Fig. II.10. Fragment of crystalline structure of $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$.

The Zr–F distance in the complex anion integrates within the interval of 1.951(2)–2.012(2) Å. An important role in forming the crystalline structure play the hydrogen bonds of N–H···F, O1w–H···F type, that establish among aniline nitrogen atoms, fluorine atoms of $[ZrF_6]^{2-}$ complex anion and crystallization water molecules.

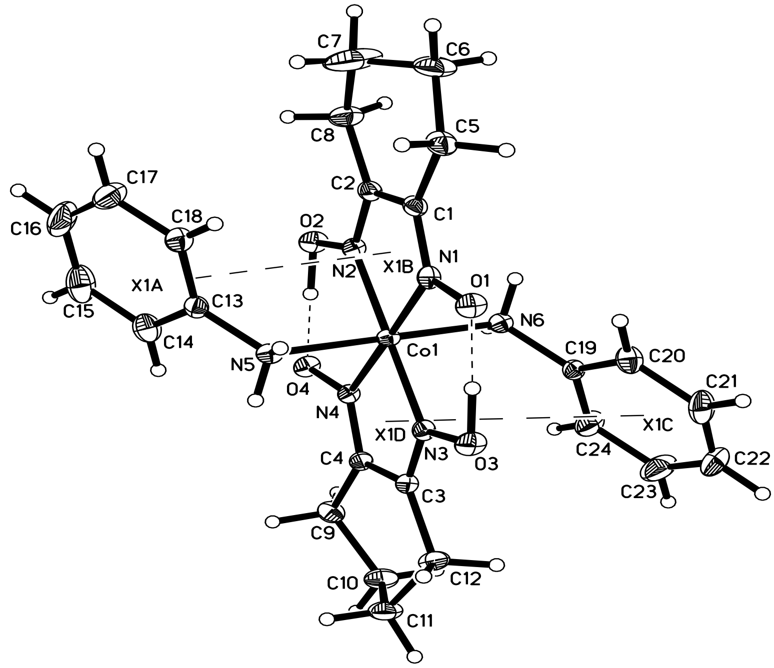


Fig. II.11. The structure of the complex cation in $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$.

The structure of $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$ compound includes: $[Co(NioxH)_2(An)_2]^+$ complex cation (Fig. II.11), $[ZrF_6]^{2-}$ centrosymmetric anion from the external spere of coordination and three molecules of crystallization water O1w, O2w, O3w. The replacement of DH^- fragment with $NioxH^-$ does not significantly influence the formation of CoN_6 coordination polyhedron and the orientation of aniline molecule. The exit of cobalt atom from plane N4 formed of nioxime atoms is equal to 0.012 Å, $(Co\text{-}N)_{NioxH^-}$, distance oscillates within the interval of 1.890(2)-1.902(2) Å, while $(Co\text{-}N)_{An}$ distance constitutes 2.012(2) and 2.013(2) Å. The aromatic rings C13-C18 and C19-C24 of aniline molecules occupy practically parallel positions as compared to the metal cycles of the equatorial plane.

Dihedral angles are respectively equal to 30.3(1) and 32.6(1)°, and the distance between their centers – 3.067(2), and 3.991(2) Å. The last values, as in the case of $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$ compound admits the existence of weak π-π interactions.

The exit of atoms C6 and C10 from the average plane of atoms C1, C2, C5, C7, C8 and C3, C4, C9, C11, C12, belonging to equatorial fragment, constitutes 0.542(5) and 0.640(3) Å, that makes them look like some distorted „semi-armchairs". We should also mention that the hexaatomic ring C1, C2, C5, C6, C7, C8 of monodeprotonate nioxime exists under two conformers, as a result of carbon atom C6 location in two positions C6 and C6'. The exit of C6' from the above described plane represents 0.18(1) Å.

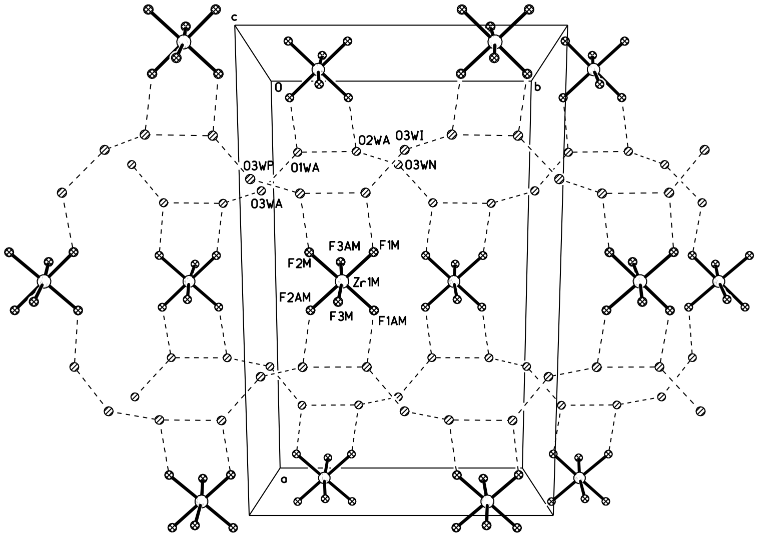


Fig. II.12. The supramolecular system of $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$.

A determining role in organizing the crystalline structure play the anion of $[ZrF_6]^{2-}$, where the Zr-F distance oscillates from 2.001(1) to 2.013(1) Å, and the three molecules of crystallization water O1w, O2w, O3w, on whose basis it is achieved the conexion between

$[Co(NioxH)_2(An)_2]^+$ complex cations through hydrogen bonds of N-H···F and C-H···F type (Fig. II.12). A special interest for this complex is the fact that complex anions and the molecules of cryatallization water form an individual supramolecular system due to a complicated system of hydrogen bonds among them. In Fig. II.12 it is exposed a fragment of this supramolecular system along direction [001].

Thus, we can state that $[ZrF_6]^{2-}$ steric voluminous anion $[ZrF_6]^{2-}$ (Zr-F distance oscillates within the interval of 1.951(2)-2.012(2) Å), shows higher possibilities in organizing the crystalline structure, as compared to other external sphere anions like: $[AlF_6]^{3-}$, $[SiF_6]^{2-}$, $[BF_4]^-$, $[BeF_4]^{2-}$, where the lengtht of bonds is shorter (oscillating within the interval of

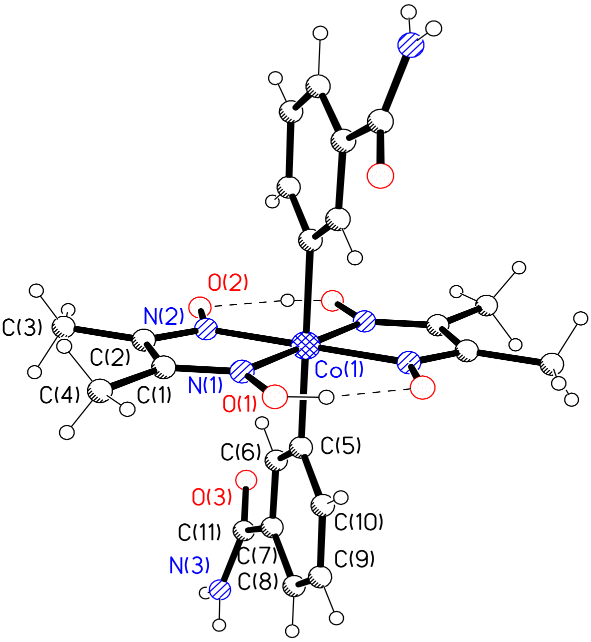


Fig. II.13. The structure of $[Co(DH)_2(Nia)_2]^+$ complex cation.

It has been synthesized and determined the structure of Co(III) compound with α-dimethylglyoxime [173] with $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$

composition where, on apical coordinates, can be found the molecules of a ligand with biological properties – nicotinamide (Nia) (Fig. II.13).

Octahedral complexes of Co(III) (Co-N(DH) 1.907(5), Co-N(DH) 1.890(5), Co-N(Nia) 1.975(4) Å) within the equatorial plane are stabilized through O-H···O (O···O 2.486 Å, O-H 0.820 Å, H···O 1.693 Å) intramolecular hydrogen bonds, bounding two α-dimethylglyoxime radicals into a pseudo-macrocyclic system. PP apical ligands of neghbouring complexes participate through amide groups in forming $R_2^2(8)$ synthon. This hydrogen bond contributes to the formation of a unidimensional chain, oriented on the crystal *x* axis. The parameters of hydrogen bonds are: N(4)···O(3) (-x, -y, 1-z) 2.831 Å, N(4)-H 0.869 Å, H···O(3)(-x, -y, 1-z) 1.979 Å. Cation chains, by means of C-H···O hydrogen bonds (donors – methyl groups of DH^- ligands, acceptors – oxygen atoms of DH^- and Nia ligands), are connected in a carcass, forming canals oriented along the crystal y-axis. In canals, are located BF_4^- anions and water molecules, connected by O(1W)-H···F 2.831 Å hydrogen bond.

Using the second hydrogen atom, amino groups of PP ligands participate as donors in the formation of hydrogen bonds with water molecules N(4)-H···O(1W) (N(4)···O(1W) 3.143 Å, N(4)-H 0.860 Å, H···O(1W) 2.2409 Å.).

The metal complexes have been tested in three concentrations – 1, 5 and 10 mg/L. In the obtained cultural liquids, both in experimental (with coordination compounds) and control variants (without coordination compounds), it was dosed the enzymatic activity specific of study strains. Amylolytic activity was determined by colorimetric method with iodine, using as a substrate the soluble amidine solution of 1%, in standard conditions of hydrolysis, and at pH=2.5 for acid stable amylases. Lipolytic activity was determined after polyvinyl alcohol action on olive oil emulsion (Oto-Iamada method) [179].

The analysis of obtained data, as a result of studying the influence of new Co(III) dioxymates on the biosynthesis of ordinary (acid labile) (Tab. II.10) and acid stable (Tab. II.11) amylases by *Aspergillus niger* 33-19 CNMN FD 02A micromycete, points out the fact that the first two substances $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot3H_2O$ and $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot2H_2O$, characterized through identical external sphere $[ZrF_6]^{2-}$ anion and different by composition of $NioxH_2$ and DH_2 ligands, show a stimulating action at the initial stage of amylase biosynthesis (on the 4-th day and at a lesser degree on the 5-th day) at their application in small concentrations – 1-5 mg/L [98, 135, 175]. The stimulating effect represents 19.98% and 15.04% for ordinary amylases, and 22.1% and 8.1% for acid stable amylases. The stimulating effect is stronger in the complex containing 1,2-cyclohexanedione dioxime radical. This can be explained by the capacity of some micromycete species to effectively assimilate and include within metabolic processes methoxylated cyclic substances. A similar action is manifested by $[Co(DH)_2(Nia)_2][BF_4]\cdot2H_2O$ substance, the stimulating effect being more obvious on the 4-th cultivation day. Complex application in the concentration of 1 mg/L increases the activity of ordinary amylases by 26.01%, and that of acid stable amylases – by 36.23% [103, 170].

Table II.10.

Modification of ordinary amylase (pH 4.7) activity of *Aspergillus niger* 33-19 CNMN FD 02A under the influence of cobalt(III) complexes with fluorinated anions (u/mL).

Coordination compound	Conc., mg/L	Activity							
		Day 4		**Day 5**		**Day 6**		**Day 7**	
		u/mL	% compared to control	u/mL	% compared to control	u/mL	% compared to control	u/mL	% compared to control
15	1	159.3	117.0	183.5	106.0	142.6	80.1	175.9	104.9
	5	163.4	**120.0**	173.1	100.0	132.5	74.5	167.5	100.0
	10	122.7	90.0	173.1	100.0	132.5	74.5	167.5	100.0
16	1	138.5	101.7	178.3	103.0	167.8	94.4	167.5	100.0
	5	150.9	110.8	199.1	115.0	112.3	63.1	175.9	104.9
	10	138.5	101.7	188.7	109.0	152.7	85.8	159.3	95.1
17	1	171.7	**126.1**	188.7	109.0	142.6	80.1	171.7	102.5
	5	159.4	117.0	188.7	109.0	157.7	88.7	184.2	109.9
	10	151.0	110.8	167.9	97.0	172.8	97.2	151.0	90.1
18	1	178.3	**130.9**	208.6	120.5	171.8	96.5	184.2	109.9
	5	178.3	**130.9**	227.4	**131.3**	171.8	96.5	192.4	114.9
	10	188.7	**138.6**	227.4	**131.3**	202.2	113.6	184.2	109.9
Control	-	136.2	100	173.1	100	177.9	100	167.5	100

Table II.11.

Modification of acid stable amylase (pH 2.5) activity of *Aspergillus niger* 33-19 CNMN FD 02A under the influence of cobalt(III) complexes with fluorinated anions (u/mL).

Coordination compound	Conc., mg/L	Activity							
		Day 4		**Day 5**		**Day 6**		**Day 7**	
		u/mL	% compared to control	u/mL	% compared to control	u/mL	% compared to control	u/mL	% compared to control
15	1	262.1	**122.1**	335.1	112.5	227.3	86.9	251.0	108.8
	5	255.8	118.8	307.5	103.2	227.3	86.9	240.6	104.3
	10	196.1	91.1	227.6	76.4	229.9	91.4	219.8	95.3
16	1	216.1	100.3	307.5	103.2	277.4	106.0	219.8	95.3
	5	227.6	105.7	321.9		171.1	65.4	251.0	108.8
	10	216.1	100.3	278.4		252.3	96.4	230.7	100.0
17	1	293.4	**136.2**	307.5	108.1	210.8	80.5	240.5	104.3
	5	270.0	125.4	278.4	93.5	246.1	94.0	245.8	106.5
	10	248.8	115.4	271.2	91.1	264.8	101.2	178.3	77.3
18	1	252.4	117.2	310.1	104.2	220.4	84.2	256.2	111.0
	5	276.6	128.4	335.1	112.5	249.4	95.3	261.3	113.3
	10	284.6	**132.2**	368.4	123.8	292.9	111.9	240.6	104.3
Control	-	284.6	100	297.8	100	261.7	100	230.7	100

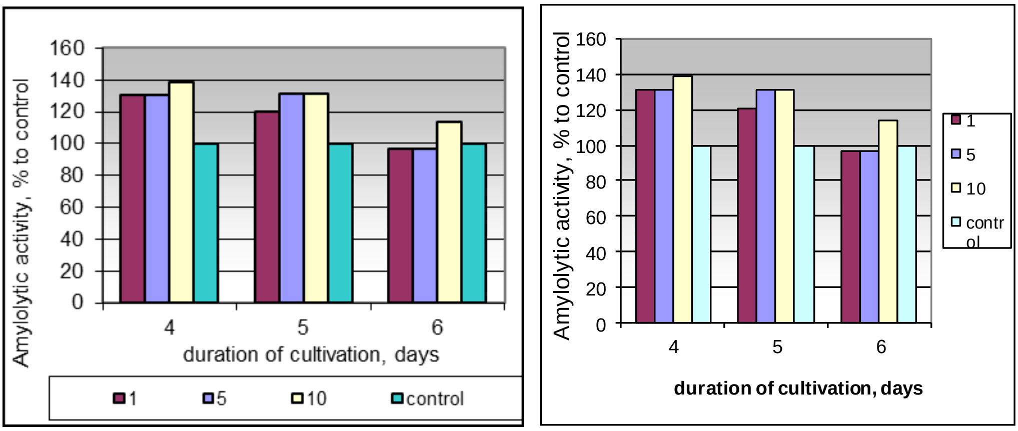
At the end of cultivation process, (days 5 and 6), the difference between experimental and control variants **15**, **16**, **17** balances. The data allow us to suppose that the tested compounds enhance the microorganism development stages (lag-phase and exponential phase) characterized by microorganism adaptation and multiplication processes, phenomenon provoking an earlier biosynthesis of amylases, fact confirmed by the considerable intensification of both ordinary and acidstable amylase biosynthesis on the 4-th cultivation day, compared to control. Microbiological research confirms the supposition by ascertaining an earlier abundance of well-developed mycelia and active sporulation in experimental variants.

The activity of both amylases is strongly modified by $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ (**18**) complex, containing the titaniun metal in the fluorinated anion composition, that distinguishes by composition from $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$ substance (**16**) containing zirconium in the fluorinated anion. The application of this complex accelerates the stationary phase establishment of strain biological cycle on days 4 and 5 in experimental variants, compared to day 6 in the control variant, reducing the technological cycle by 48 hours, fact that presents economical advantages and significantly increases enzymatic biosynthesis. The maximum of acid labile amylase activity in experimental variants (metal complex concentration of 5 and 10 mg/L) constitutes 227.36 u/mL compared to 177.89 u/mL in the control variant; of acid stable amylases – 368.35 u/mL in the experimental variant, compared to 297.79 u/mL – in the control variant. Thus, the stimulating effect represents 27.8% and 23.69% for standard and acid stable amylases respectively. The activity of amylases in this variant keeps superior to the control variant during the whole cultivation period, fact confirming that this complex stimulates microorganism activity and simultaneously generates the „de novo" biosynthesis of enzymes.

As for the similar internal sphere composition of compounds **16** and **18** – $[Co(DH)_2(An)_2]_2[ZrF_6]$, $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$, marked by complex anions difference – $[ZrF_6]^{2-}$ and $[TiF_6]^{2-}$ – we can conclude that the different action of this compound on development processes and enzymatic synthesis of *Aspergillus niger* 33-19 CNMN FD 02A is marked by the properties of $[TiF_6]^{2-}$ complex anion. Here we can note the anion influence on the effect exerted by complexes. The increase of enzymatic activity in some experimental variants on day 7 is provoked by the microorganism secondary growth. Under the microscope, at this stage, can be watched the growth of new conidia and chains of young mycelium.

Following research, there were selected the optimal conditions for applying cobalt(III) dioximates containing fluorine to enhance the biosynthesis of ordinary and acid stable amylases by the fungal strain of *Aspergillus niger* 33-19 CNMN FD 02A – producer of exocellular amylases: at the use of $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$; $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$; $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$ metal complexes as stimulators; concentration constituting 1-5 mg/L, cultivation duration – 5 days; for $[Co(DH)_2(An)_2]_2$ $[TiF_6]\cdot 2H_2O$ compound – concentration constitutes 5-10 mg/L, cultivation duration 4-5 days, depending on applied concentration. The process takes place in standard cultivation conditions established for *Aspergillus niger* 33-19 CNMN FD 02A fungal strain in submerse culture.

The research results were used to develop a proceeding of oriented microbial synthesis of amylases. The proceeding consists in: introducing in the standard cultivation medium of *Aspergillus niger* 33-19 CNMN FD 02A in submerse culture of $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ coordination compound, in the concentration of 5-10 mg/L. The compound is added to the medium after autoclaving, immediately before seeding the microorganism, in order to avoid damaging the complex structure. The proceeding provides the increase of strain amylolytic activity by 23.7-28.9%, as compared to control, and the reduction of technological cycle by 24-48 hours [103, 170, 174].



A **B**

Fig. II.14. Modification of activity of amylases synthesized by *Aspergillus niger* 33-19 CNMN FD 02A micromycete:
A Modification of ordinary amylase (pH 4.7) activity under the influence of $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ complex.
B Modification of acid stable amylase (pH 2.5) activity under the influence of $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$ complex.

The influence of cobalt(III) dioximates with fluorine was also studied on the process of enzyme genesis in the micromycete strain of Rhizopus arrhizus Fischer CNMN FD 03L – lipase active producer [86, 88, 168]. The data obtained after determiningthe lipolytic activity in dynamics, in the strain cultural liquid on cultivation days 1, 2 and 3 are exposed in Tab. II.12.

In compounds **15-17**, the stimulating effect manifests on the first cultivation day, the enzymatic activity in the variants of media with coordination compounds in concentrations of 5-10 mg/L, being by 5.5-11.1% superior to control. The maximum biosynthesis, for all compounds, records on the second cultivation day, constituting 122.2-166.6% compared to control and coinciding with the period of maximum biosynthesis in the control variant.

Table II.12.

Modification of *Rhizopus arrhizus* Fischer CNMN FD 03L micromycete lipolytic activity under the influence of cobalt(III) complexes.

Coord. comp.	Conc., mg/L	Lipolytic activity, u/mL					
		Day 1		Day 2		Day 3	
		u/mL	% compared to control	u/mL	% compared to control	u/mL	% compared to control
15	1	54625	105.5	68750	122.2	45000	100
	5	54625	105.5	68750	122.2	45000	100
	10	57500	111.1	68750	122.2	35000	77.8
16	1	51750	100	75000	133.3	35000	77.8
	5	57500	111.1	93750	166.6	45000	100
	10	57500	111.1	81250	144.4	35000	77.8
17	1	51500	100	68750	122.2	35000	77.8
	5	57500	111.1	87500	155.5	45000	100
	10	57500	111.1	68750	122.2	35000	77.8
18	1	46000	88.9	75000	133.3	40000	88.9
	5	51750	100	75000	133.3	45000	100
	10	51750	100	75000	133.3	45000	100
Control	-	51750	100	56250	100	45000	100

Different from *Aspergillus niger* 33-19 CNMN FD 02A strain, *Rhizopus arrhizus* Fischer CNMN FD 03L doesn’t reduce the development cycle, fact confirmed additionally by microscopic researches that don’t record modifications in the phases of culture development. This can be explained by the short development cycle of *Rhizopus arrhizus* Fischer CNMN FD 03L strain – 2 days, unlike *Aspergillus niger* 33-19 CNMN FD 02A strain, whose development cycle constitutes 6 days. The highest activity was recorded in complexes **16** and **17**, in the variants with the concentration of 5 mg/L, constituting respectively 93750 u/mL and 87500 u/mL, as compared to 56250 u/mL in the control variant, fact presenting an increase of 66.6% and 55.5%.

Complex **18** manifests a different action mechanism on the process of strain enzyme genesis as compared to other complexes. The curve of fungal life cycle on the medium containing this complex repeats the curve on the control medium, the difference consisting in lipase maximum biosynthesis on the second cultivation day.

Irrespective of concentration in the applied diapason of this complex – 1, 5, or 10 mg/L, enzymatic activity constitutes 75000 u/mL as related to 56250 u/mL in the control variant, or by 33.3% superior to control. This different behavior can be attributed to the complex cation with titanium from the external sphere of the compound.

During research on establishing the components of complexes, including the nature of the metal causing the stimulation effect of biological activity in fungal strains, it was tested the influence degree of a group of oxime complexes and nicotinamide ligands: $[Fe(DH)_2(Nia)_2]$ (**19**), $[Fe(DH)_2(Dena)_2]$ (**20**), $[Co(DH)_2(Nia)_2]Cl$ (**21**), $[Co(DH)_2(Dena)_2]Cl$ (**22**), $[Co(DH)_2(Dena)_2][BF_4]$ (**23**) on biosynthetic processes in *Aspergillus niger* (USA MV) CNMN FD10 strain [87, 169].

The obtained data highlight the differentiated effect of Fe(II) dioximates with nicotinamide and N,N′-diethylnicotinamide on the biosynthesis of cellulosic complex components of *Aspergillus niger* CNMN FD10 strain. The results showed in tables II.13 and II.14 reflect an obvious stimulation of endocluconase activity by $[Fe(DH)_2(Nia)_2]$ compound, that constitutes 8.70-8.56 u/mL on cultivation days 8 and 9, compared to 7.20-7.99 u/mL, in the same period, in the control variant; and of xylanase activity – by $[Fe(DH)_2(Dena)_2]$ compound, the enzymatic activity in the experimental variant constituting 147 u/mL compared to 120 u/mL in the control variant.

Table II.13.

Modification of *Aspergillus niger* CNMN FD10 cellulolytic activity under the influence of Co and Fe dioximates with nicotinamide and N,N′-diethylnicotinamide, in dynamics.

Complex	Concentr., mg/L	Endoglucanases activity, u/mL		
		7 days	8 days	9 days
$[Fe(DH)_2(Nia)_2]$	1	**7.61**	**8.70**	**8.56**
	5	5.84	**8.70**	7.42
	10	5.84	7.07	7.42
$[Fe(DH)_2(Dena)_2]$	1	**8.06**	**8.16**	7.05
	5	6.52	7.88	7.33
	10	6.39	6.80	7.05
$[Co(DH)_2(Nia)_2]Cl$	1	**7.34**	**7.88**	7.62
	5	6.39	6.25	6.66
	10	4.35	5.44	6.09
$[Co(DH)_2(Dena)_2]Cl$	1	2.85	7.34	5.52
	5	5.30	**7.70**	5.71
	10	5.98	**7.80**	7.42
$[Co(DH)_2(Dena)_2][BF_4]$	1	5.84	7.07	6.28
	5	4.86	7.21	6.28
	10	5.98	7.16	6.85
Control	0	7.14	7.20	7.99

Both compounds manifested as inhibitors of β-glucosidases. Co(III) dioximates with the same ligands, containing additionally anions of Cl^- and $[BF_4]^-$ in the external sphere, in small concentrations – 1.0-5.0 mg/L, practically do not influence endocluconase and xylanase activity and display inhibiting action towards β-glucosidases.

Table II.14.

Modification of *Aspergillus niger* CNMN FD10 xylanase activity under the influence of Co and Fe dioximates with nicotinamide and N,N′-diethylnicotinamide, in dynamics.

Metalocomplex	Concentr., mg/L	Xylanase activity, u/mL		
		7 days	8 days	9 days
$[Fe(DH)_2(Nia)_2]$	1	121.85	89.21	88.12
	5	121.85	104.48	73.44
	10	115.32	95.74	81.60
$[Fe(DH)_2(Dena)_2]$	1	**147.98**	110.97	97.92
	5	121.85	108.80	86.49
	10	117.50	-	94.65
$[Co(DH)_2(Nia)_2]Cl$	1	121.85	82.68	106.08
	5	115.32	100.09	99.55
	10	97.92	91.39	94.65
$[Co(DH)_2(Dena)_2]Cl$	1	95.74	108.80	65.28
	5	100.09	**121.85**	88.12
	10	100.09	117.50	88.12
$[Co(DH)_2(Dena)_2][BF_4]$	1	108.80	115.32	78.33
	5	106.62	**120.38**	81.60
	10	97.92	104.48	94.65
Control	0	121.85	106.62	96.30

The complex of $[Co(DH)_2(INia)_2][BF_4]$ (**24**), in concentrations of 1 and 5 mg/L, manifests stimulating action on the pectolytic activity of *Penicillium viride* CNMN FD 04P [145], exceeding the control variant by 16.4-24.0% (Table II.15).

Table II.15.

Modification of *Penicillium viride* CNMN FD 04 pectolytic activity under the influence of $[Co(DH)_2(INia)_2][BF_4]$ complex, u/mL.

Concentr., mg/L	Day 3		Day 4	
	PA, u/mL	%, compared to control	PA, u/mL	%, compared to control
1	368.33	98.0	529.02	90.1
5	457.97	121.9	683.22	**116.4**
10	386.03	102.8	727.55	**124.0**
Control	357.64	100	586.84	100

Another series of tested dioximates contain the anion of $[TiF_6]^{2-}$: $[Co(NioxH)_2(Thio)_2]_2[TiF_6]\cdot 3H_2O$ (**25**), $[Co(DH)_2(Thio)_2]_2[TiF_6]\cdot 2H_2O$ (**26**), $[Co(DH)_2(Py)_2]_2[TiF_6]\cdot 2H_2O$ (**27**).

The compound $[Co(NioxH)_2(Thio)_2]_2[TiF_6]\cdot 3H_2O$ is formed of two crystallographically independent cations, $[TiF_6]^{2-}$ polyfluoro metallic anion and three water molecules [171]. The equatorial plane of the complex cation is occupied by two anions of 1,2-cyclohexanedione dioxime, connected through intramolecular hydrogen bonds, with the distance between donors·acceptors comprised within the interval of 2.500-2.567 Å. The length of these bounds are higher (~0.1-0.02 Å) than in the case of the similar complex containing the anion of $[ZrF_6]^{2-}$. The exit of cobalt atom from the equatorial plane N4 does not surpass 0.015 Å. Thiocarbamide molecules are located differently within complex cations, towards the equatorial plane.

The S1C13N5N6 fragment forms an angle of 62.06° with Co1N3N4C7C12 plane, while S2C14N7N8 forms an angle of 22.75° with Co1N1N2C1C6 plane. These orientations are mostly influenced by the position of $[TiF_6]^{2-}$ anion that forms multiple hydrogen bonds with NH_2 groups. The parallel orientation of thiocarbamide fragment towards the equatorial plane leads to the achievement of π-π interactions between the latter and the metal cycle of the equatorial plane (distance

C14···X1=3.040 Å, where X1 is the center of equatorial plane metal cycle; Fig. II.15).

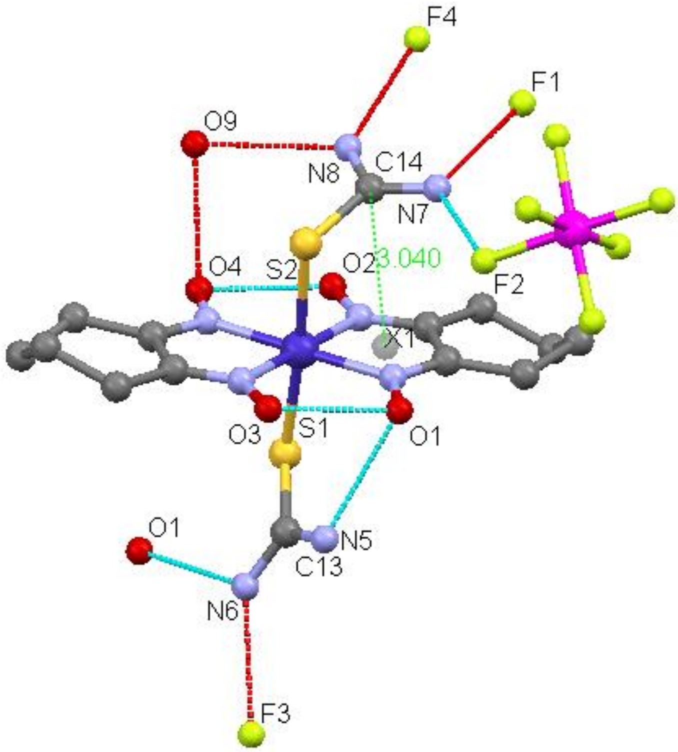


Fig. II.15. Orientation of thiocarbamide molecules in $[Co(NioxH)_2(Thio)_2]_2[TiF_6]\cdot 3H_2O$ complex due to intra- and intermolecular hydrogen bonds.

Perpendicularly oriented thiocarbamide forms an intramolecular hydrogen bond with the oxygen of N5–H5D···O1 (2.930 Å) oxime group, while with the other NH_2 group, it forms an intermolecular hydrogen bond with the oxygen of the oxime group of a different complex cation. Therefore, this allows the rotation of S1C13N5N6 plane under the angle of 32.39° towards Co1S1C13 plane. In case of parallel orientation, the lack of hydrogen bonds with oxime groups oxygen keeps these planes approximately mutually perpendicular (the angle between Co1S2C14 and S2C14N7N8 planes being 85.10°).

The same effect can be noticed as regards the second cation within $[Co(NioxH)_2(Thio)_2]_2[TiF_6]\cdot 3H_2O$. The angles formed between Co2S4C28 and S4C28N15N16; Co2S3C27 and S3C27N13N14 planes are equal to 29.11° and 88.69° respectively. If we compare the size of these angles to those of the similar complex $[Co(NioxH)_2(Thio)_2]_2[ZrF_6]\cdot 3H_2O$, it can be noticed that some vary by about 13°. Analyzing the hydrogen bonds that orient the thiocarbomide molecule in such a position, it can be noticed that in case of $[Co(NioxH)_2(Thio)_2]_2[TiF_6]\cdot 3H_2O$ compound, the N–H···O bond between the latter and the oxime group is higher (2.884 Å), as compared to that of $[Co(NioxH)_2(Thio)_2]_2[ZrF_6]\cdot 3H_2O$ (2.848 Å) complex, while the hydrogen bonds with polyfluorometallic anion are shorter (2.824 and 2.868 Å within $[Co(NioxH)_2(Thio)_2]_2[TiF_6]\cdot 3H_2O$; 2.852 and 2.897 Å within $[Co(NioxH)_2(Thio)_2]_2[ZrF_6]\cdot 3H_2O$). This leads to a difference of 13° of the torsion angle. Therefore, it can be considered that the orientation of molecules of axial positions containing functional groups, that would form hydrogen bond, depends both on the anion of the external sphere and its volume.

In octahedral $[TiF_6]^{2-}$ anions, the Ti-F lengths vary within the interval of 1.824-1.873 Å, being in concordance with literature data concerning this anion [172]. All the six fluorine atoms participate in the formation of hydrogen bonds, therefore the anion has a determining role in the formation of crystalline network within the complex.

The data referring to the effect produced by the study compounds on the biosynthetic activity of *Penicillium viride* CNMN FD 04 P micromycete are exposed in table II.16.

Following the obtained results [90, 95], it is obvious that effect vary depending on both compound structure and applied concentration. It has been found that for all tested compounds, minor concentrations of 1 and 5 mg/L are the most favorable. The concentration of 10 mg/L is inefficient, hence in the given variants the effect is neutral, or even inhibiting (in the case of $[Co(DH)_2(Thio)_2]_2[TiF_6]\cdot 2H_2O$ compound, activity diminished by about 20%).

Table II.16.

Modification of *Penicillium* viride CNMN FD 04 pectolytic activity under the influence of Co coordination compounds with fluorinated anions.

Coordination compound	Conc. mg/L	3-rd cultivation day		4-th cultivation day	
		PA, u/mL	%, compared to control	PA, u/mL	%, compared to control
25	1	445.37	97.3	894.37	**140.7**
	5	474.28	103.6	669.88	105.4
	10	437.98	95.7	654.40	102.9
26	1	491.31	107.3	631.17	99.3
	5	453.72	99.1	816.96	**128.5**
	10	354.46	77.4	507.32	79.8
27	1	443.12	96.8	727.94	114.5
	5	418.39	91.4	813.09	**127.9**
	10	411.32	89.8	648.98	102.0
Control	0	457.90	100	635.82	100

The maximum increase of pectolytic activity (40.7% compared to control) was recorded while including compound **25** in the cultivation medium. Intensification of pectinase synthesis was recorded in the variants with the following two compounds included in the investigation, constituting 28.5% as compared to control, in the case of compound **26** (conc. 5 mg/L), and about 15 and 28% in the variant with compound **27**, in the concentration of 1 and 5 mg/L, correspondingly. The composition of complexes **26** and **27** differ by dioxime nature, whence it results that in the given case, nioxime manifests a higher influence than dimethylglyoxime in the process of stimulating pectolytic activity.

It has been established that the tested compounds do not modify the development cycle of *P. viride* micromycete, the maximum pectolytic activity being recorded on the 4-th cultivation day, simultaneously with the control variant. A very different picture appears in the case of given compounds influence on amylolytic activity of *Aspergillus niger* 33-19 CNMN FD 02A micromycete, thus confirming the fact that effect varies depending on both coordination compounds composition and microorganism properties, and the type of synthesized enzymatic system. The data included in table II.17, despite the pursued effect in the series of experiments on *Penicillium viride* strain, show that all tested compounds intensify the process of amylase synthesis in *Aspergillus niger* 33-19 CNMN FD 02A micromycete, effect amplifying in the range: **25** < **26** < **27**.

The stimulating influence is pursued especially in the case of ordinary amylases (pH 4.7), activity increasing from 10% to about 45%, along with the growth of applied concentration. Adjacently, metal complexes accelerate by 24 hours the manifestation of maximum biosynthesis. Thus, on the 5-th cultivation day, the amylolytic activity (at pH 4.7) of samples cultivated in the presence of metal complexes (in the concentration de 10 mg/L) varies between 266.73-292.77 u/mL, compared to 202.25 u/mL in the control variant on the day of maximum biosynthesis (6-th cultivation day), exceeding the control by 31.9-44.8%. On the 6-th cultivation day, the level of amylolytic activity in experimental and control samples equalize.

More favorable for *Aspergillus niger* 33-19 CNMN FD 02A culture is the compound of $[Co(DH)_2(Py)_2]_2[TiF_6]\cdot 2H_2O$ that, in all tested concentrations, ensures the content increase of both ordinary amylases (pH 4.7) – by 17.9-44.8%, and acid stable amylases (pH 2.5) – by 18.7-28.2%. Analyzing the results for Co(III) dioximates by composition of anions, it was established that the biological activity of the given compounds decreases in the following order: $[BF_4]^-$ > $[TiF_6]^{2-}$ > $[ZrF_6]^{2-}$ [103].

Table II.17.

Modification of *Aspergillus niger* 33-19 CNMN FD 02A amylolytic activity under the influence of Co(III) coordination compounds with fluorinated anions.

Coordination compound	Conc. mg/L	Amylolytic activity at pH 4.7				Amylolytic activity at pH 2.5			
		Day 5		Day 6		Day 5		Day 6	
		u/mL	%, compared to control on day 6	u/mL	%, compared to control on day 6	u/mL	%, compared to control on day 6	u/mL	%, compared to control on day 6
25	1	223.31	110.4	178.97	88.5	165.70	59.1	267.86	95.5
	5	238.50	117.9	198.02	97.9	173.96	62.0	276.33	98.5
	10	266.73	131.9	225.53	111.5	248.30	88.5	314.43	112.1
26	1	234.16	115.8	219.18	108.4	285.47	101.7	312.31	111.3
	5	246.98	122.1	206.48	102.1	312.40	111.3	278.45	99.3
	10	280.44	138.7	204.37	101.1	347.84	124.0	253.05	90.2
27	1	238.5	117.9	242.47	119.9	332,96	118.7	291.15	103.8
	5	273.24	135.1	229.77	113.6	345.47	123.1	253.05	90.2
	10	292.77	144.8	207.07	107.3	359.65	128.2	202.25	72.1
Control	0	172.63	-	202.25	100	262.38	-	280.56	100

The study of the influence of some coordination compounds of cobalt, copper and zinc, based on oxime ligands, on exocellular hydrolase biosynthesis in the mycelial fungi strains highlighted the differentiated effect of tested compounds on hydrolase biosynthesis according to taxon and synthesized enzymatic system [178]. In order to pinpoint the dependence of coordination compounds biological effect on fungal systematic position and enzyme systems synthesized in different researches, there were entailed micromycetes from different genera, with different enzymatic systems: *Aspergillus niger* CNMN FD 10 – cellulase and xylanase producer, *Trichoderma koningii* CNMN FD 15 and *Fusarium gibbosum* CNMN FD 12 – protease producer, and from different genera with similar enzymatic systems – *Trichoderma koningii* and *Fusarium gibbosum* – protease producers. Researches were achieved on nutrient media and optimized cultivation regimes background, ensuring optimal conditions both for culture growth and development and for maximum biosynthesis of enzymes.

Within investigation, there have been applied classical and modern microbiological and biological methods accepted in enzymology.

The biomass was separated from the cultural liquid through mechanical filtration. The activity of acid (pH 3.6) and neutral (pH 7.4) exocellular proteases was determined according to Willstatter method, based on gelatin degradation to polypeptides and amino acids, with subsequent determination of free carboxylic groups.

The activity of cellulase complex and xylanases was determined by enzymes action on corresponding substrates: β-glucosidase – on 4-nitrophenyl-β-D-glucopyranoside, endoglucanase – on Na-carboxymethyl cellulose, xylanase – on oat xylan, subsequently dosing reducing sugars according to the Somogy-Nelson colorimetric method [179, 180].

At the first research stage there were included the *Trichoderma koningii* and *Fusarium gibbosum* – protease producer strains from different genera with similar enzymatic systems.

The strain of *Trichoderma koningii* Oudemans CNMN FD 15 distinguishes through the capacity to synthesize all three types of proteases: acid (pH 3.6), neutral (pH 7.4) and basic (pH 9.2). The enzymatic complexes synthesized by a single producer manifests a more obvious action synergism, fact determining the intensity of substrate hydrolysis processes, while the component enzymes have more homogenous traits, facilitating their practical use [183,148].

The strain of *Fusarium gibbosum* CNMN FD 12 synthesizes as profile enzymes acid (pH 3.6) and neutral (pH 7.4) proteases and as secundary components, in significant amounts, cellulases and xilanases [180, 149].

The research started by studying the influence of oxime based coordination compounds of Co(III), Cu(II) and Zn(II) on protease biosynthesis by *Trichoderma koningii* Oudemans CNMN FD 15 fungi strain.

The researches, aimed at establishing the effect of metalcomplexes on protease biosynthesis by *Trichoderma koningii* Oudemans CNMN FD 15, included cobalt coordination compounds with selen carbamide $[Co(DH)_2(Seu)_{7/4}(Se\text{-}Seu)_{1/4}]_2[TiF_6]\cdot H_2O$ (**28**), copper coordination compounds with disulfanilamideglyoxime $[Cu(DSamH_2)_3]SO_4$ (**29**) and zinc coordination compounds with 4,4-bipyridyl – $[Zn_2(CH_3COO)_4(NioxH_2)_2bpy(H_2O)_2]$ (**30**). The structural characteristics of complexes are exposed below.

Within UV-Vis spectrum of complex **28** in 200-400 nm region there are 2 absorption bands: the first demonstrates dioxime presence in the equatorial plane, conditioned by π-π^* transfer in $Co(DioxH)_2$ grouping, the second confirms the the presence of coordinated selen carbamide. Within IR spectrum of compound **28**, the presence of $\nu(CN)=1574$, $\nu(NO)=1236$ and 1087, $\nu(Co–N)=514$ and 420 cm^{-1} absorption bands indicates on the coordination of dimethylglyoxime monoanions to the central atom.

Within NMR ^1H spectrum of complex **28**, $\delta(CH_3)$ signals shift from region 1.96 ppm to the weak field direction at 2.2-2.4 ppm, fact determined by electronic density transfer from ligand (dimethylglyoxime) to metal. Singlet presence within the complex spectrum in the regions of methyl groups shows that these groups are magnetically equivalent, and the studied complex has a *trans*-configuration. The signals in region 8.10-8.45 ppm correspond to amine groups of selenocarbamide. Within NMR

^{19}F spectrum it is present the signal characteristic to δ^{19}F($[TiF_6]^{2-}$) anion of the external sphere at 72 ppm. The presence of a single signal within the spectrum indicates on the fact that all fluorine atoms are magnetically equivalent and complex anions are stable in solution.

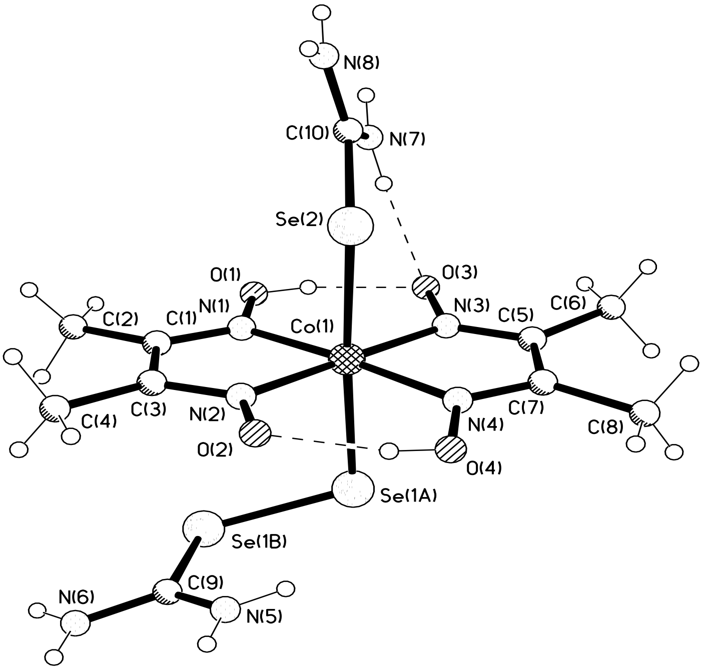


Fig. II.16. The structure of $[Co(DH)_2(Seu)(Se\text{-}Seu)]^+$ complex cation in **28**.

After deciphering the crystal structure [176], it has been established that in **28** there are two types of complex cations: $[Co(DH)_2(Seu)_2]^+$ and $[Co(DH)_2(Seu)(Se\text{-}Seu)]^+$ (Fig. II.16.) in a ratio of 3:1. We consider that Se-Seu ligand is formed in the system by elementary selen, that subsequently joins the selenocarbamide molecule.

Within the central atom, in the equatorial plane (Fig. II.16.), there are four coordinating nitrogen atoms from two monoanions of dimethylglyoxime bound through intramolecular hydrogen bonds. In positions 1,6, coordinate selenium atoms of axial ligands, the external shere being constituted of $[TiF_6]^{2-}$ anions and water molecules.

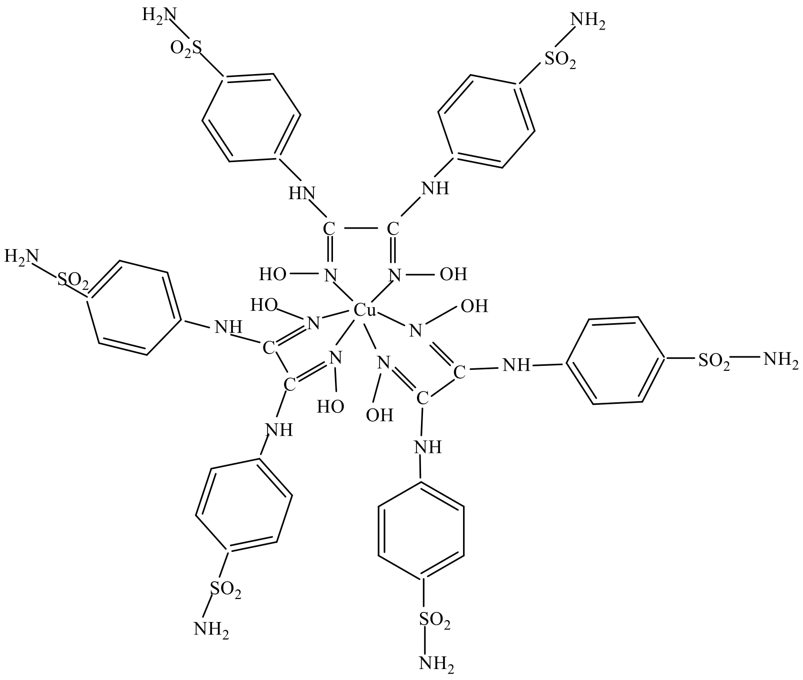


Fig. II.17. The structure of $[Cu(DSamH_2)_3]^{2+}$ complex cation in **29**.

Complex **29** is formed of $[Cu(DSamH_2)_3]^{2+}$ complex cation that, in the central atom, coordinate in a chelation way three molecules of disulfanilamideglyoxime, while in the external shere can be found sulphate anions. In this *tris*-dioximic complex, the oxime groups of $DsamH_2$ ligand do not deprotonate. The complex generator display the coordination number 6, a rare case characteristic of copper (Fig. II.17).

The influence of coordination compounds on micromycete proteolytic activity was dynamically evaluated during the 8-th and the 9-th cultivation days, days of protease maximum biosynthesis in conditions of producer classical cultivation (Tab. II.18 and II.19).

Within complex **30**, each Zn^{2+} cation is hexacoordinated on the account of three atoms of oxygen and three atoms of nitrogen, into an octahedral geometric sphere (Fig. II.18).

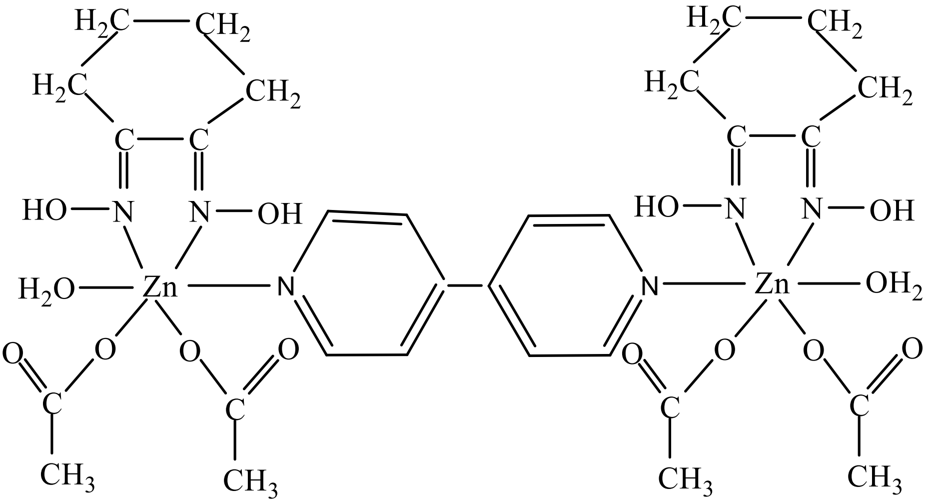


Fig. II.18. The structure of $[Zn_2(CH_3COO)_4(NioxH_2)_2bpy(H_2O)_2]$ binuclear molecule.

The basal plane of coordination polyhedron is formed of $NioxH_2$ bidentate molecules and two monodentate acetate anions. The apical position is occupied by a water molecule and the bpy bidentate ligand, that fulfills the function of bridging ligand. The neutral molecule of $NioxH_2$ coordinates in a typically bidentate way, by means of oxime nitrogen atoms, thus forming a five-member chelated cycle with the complex generator. Two acetate anions coordinate in a monodentate way, occupying the other two positions of the basal plane. The IR spectrum of compound **30** includes bands characteristic of oxime group: υ(O-H) valence oscillations specific of 1,2-cyclohexanedione dioxime were recorded at 3192 cm^{-1}; υ(N-O) bands were noticed at ~1259, 1217, 949 cm^{-1}.

The band at 1600 cm^{-1} can be attributed both to υ(CC) valence oscillations, and to υ(C=N) of bpy coordinated ligand. The band of region 1491 cm^{-1} corresponds to υ_s(C=C) valence oscillations of the bridging ligand. Within the interval 613-666 cm^{-1}, there have been observed peaks corresponding to γ(CCC) and γ(CNC) valence oscillations belonging to the cyclic ligand.

The presence of acetate residues within complexes is demonstrated by $\delta(CH_3)$ ~ at 1460, 1434, 1353 and $\rho_r(CH_3)$ at 1040 cm^{-1}oscillations.

Besides, there were detected υ(COO) valence oscillations within regions 1550, 1409 cm^{-1}, and π(COO) in region 613 cm^{-1}. The bands at 3003, 2948 cm^{-1} were attributed to υ(CH) valence oscillations. There were also observed υ(CC) oscillations in region 930 cm^{-1}. M-O-C metal-ligand oscillations were recorded at ~1980 cm^{-1}, υ(M-O)+υ(C-C) at ~518 cm^{-1}, and υ(M-N) at ~420 cm^{-1}.

The influence of coordination compounds on the proteolytic activity of *Trichoderma koningii* Oudemans CNMN FD 15 micromycete has been dynamically evaluated during the 8-th and the 9-th cultivation days, days of protease maximum biosynthesis in classical conditions of producer cultivation (Tab. II.18 and II.19).

According to the data exposed in the table, cobalt compound with dimethylglyoxime and selenocarbamide – $[Co(DH)_2(Seu)_{7/4}(Se\text{-}Seu)_{1/4}]_2[TiF_6]\cdot H_2O$ (**28**) in concentrations of 10-15 mg/L increases the biosynthesis of acid proteases by 37.1-122.0%, and that of neutral proteases by 13.8-44.1%, enzymatic activity constituting 3.53-2.18 u/ml compared to 1.59 u/ml, and 3.38-4.28 u/ml compared to 2.97 u/ml in the control variant, respectively, for acid and neutral proteases. The maximum activity recorded in the concentrations of 5-10 mg/L, while the maximum synthesis was ensured by the superior concentration. On the 9-th cultivation day the biosynthesis of acid proteases records significantly low values as compared to control variant.

Table II.18.

The influence of oxime-based coordination compounds of zinc, cobalt and copper on the activity of *Trichoderma koningii* Oudemans CNMN FD 15 acid proteases.

Coord. comp.	Conc., mg/L	The activity of acid proteases (pH 3.6)					
		Day 8		Day 9		Day 10	
		u/mL	% compared to control	u/mL	% compared to control	u/mL	% compared to control
28	5	0.42	53.2	0.84	52.8	0.08	19.0
	10	0.67	84.8	3.53	**222**.0	0.50	119.0
	15	0.42	53.2	2.18	137.1	0.42	100
29	5	1.76	222.8/110.7*	0.92	57.9	0.59	140.5

	10	1.93	**244.3/121.4***	0.08	5.0	0.42	100
	15	0.84	106.3	0.0	0.0	0.0	-
30	5	1.92	**243.0/110.2***	1.26	79.2	0.42	100
	10	1.68	212.6/ 105.7*	1.51	94.9	0.37	88.1
	15	1.43	181.0	0.50	31.4	0.08	19.0
Control	-	0.79	100	1.59	100	0.42	100

*compared to control variant of the day/ to the maximum value of control variant (day 9)

The complex doesn't modify the fungal life cycle: the enzymatic biosynthesis maximum manifests on the 9-th cultivation day and coincides with the maximum of protease biosynthesis in the control variant.

The enzymatic activity of experimental variants below the level of control variant activity, on the 8-th cultivation day, shows on the necessity to adapt the culture to medium new conditions, fact leading to retention of cell multiplication [182].

Table II.19.

The influence of oxime-based coordination compounds of zinc, cobalt and copper on the activity of *Trichoderma koningii* Oudemans CNMN FD 15 neutral proteases.

Coord. comp.	Conc., mg/L	The activity of neutral proteases (pH 7.4)					
		Day 8		Day 9		Day 10	
		u/mL	% compared to control	u/mL	% compared to control	u/mL	% compared to control
28	5	2.15	94.7	2.90	97.6	1.19	52.4
	10	2.27	100.0	3.38	113.8	0.89	39.2
	15	2.27	100.0	4.28	**144.1**	1.77	77.9
29	5	4.04	**177.9**	4.53	152.5	3.15	138.8
	10	3.03	133.5	2.64	88.9	3.27	144.1

	15	2.01	88.5	2.52	84.8	2.64	116.3
30	5	5.04	**222.0**	1.89	63.6	1.52	66.9
	10	3.21	141.4	1.89	63.6	1.98	87.2
	15	2.90	127.7	1.52	51.2	1.89	83.3
Control	-	2.27	100	2.97	100	2.27	100

In the case of Cu coordination compound with disulphanilamideglyoxime, the maximum of acid protease biosynthesis (1.76-1.93 u/mL) records on the 8-th cultivation day, exceeding the maximum value (1.59 u/mL) of control variant on the 9-th cultivation day by 110.7-121.4%.

As for the biosynthesis of neutral proteases, the supplementation of cultivation medium with Cu(II) binuclear compound, doesn't modify the manifestation term of enzyme maximum biosynthesis. The maximum (4.53 u/mL) of neutral protease activity manifests similarly to control variant – on the 9-th cultivation day, exceeding the level of control by 52.5%. At supplementation of micromycete nutrient medium with Cu(II) complex in concentrations of 5-10 mg/L, the activity of neutral proteases is superior to control during the whole cultivation period, days 8-10, fact characterizing the complex as a specific stimulator of neutral proteases in *Trichoderma koningii* Oudemans. The occurance of neutral proteases in the culture medium, in bigger amounts, at a more precocious term, indicates on the mechanism of increasing biosynthesis through intensification of culture development and multiplication in the presence of the metal complex within the cultivation medium.

The binuclear complex of Zn(II) manifests an obvious biostimulating influence on acid and neutral proteases and on the acceleration of biosynthesis maximum manifestation, fact indicating on the intensification of development and culture multiplication by the metal complex.

On the 8-th cultivation day, in all tested concentrations, the activity of both acid and neutral proteases is superior to the activity of the day control variant and to that of the day of maximum biosynthesis for control, constituting 1.92; 1.68 and 1.43 u/mL towards 0.79 u/mL in the control variant of the day and 1.59 u/mL (on the 9-th day) for acid proteases, and respectively 5.04, 3.21, 2.90 u/mL towards 2.27 u/mL and 2.97 u/mL in the control variant on days 8 and 9, for neutral proteases. The maximum increase for acid proteases represents 110.2%, and for neutral proteases – 69.7%, being ensured by the compound concentration of 5 mg/L.

Considering the fact that *Trichoderma koningii* Oudemans CNMN FD 15 and *Fusarium gibbosum* CNMN FD 12 micromycetes synthesize similar enzyme complexes, in the research with *Fusarium gibbosum* CNMN FD 12 strain, it was used the same group of oxime-based coordination compounds of Co (**28**), Cu (**29**) Zn (**30**). The influence of coordination compounds on micromycete proteolytic activity was evaluated in dynamics, during cultivation days 4-6, that corresponds to the period of maximum biosynthesis manifestation for *Fusarium gibbosum* in conditions of classical cultivation [179] (Tab. II.20 and II.21).

Table II.20.

The influence of oxime-based coordination compounds of Co(III), Cu(II), Zn(II) on the activity of acid proteases in *Fusarium gibbosum* CNMN FD 12.

Coordination compound	Con. mg/L	The activity of acid proteases (pH 3.6)					
		Day 4		Day 5		Day 6	
		u/mL	%, compared to control	u/mL	%, compared to control	u/mL	%, compared to control
28	5	0.17	33.3	4.28	154.5	0.67	57.1
	10	0.76	150.0	4.70	**169.7**	0.42	35.7
	15	0.08	16.7	4.54	163.6	0.59	50.0
29	5	0.25	50.0	4.28	**154.5**	2.02	171.4
	10	0.59	116.7	2.69	97.0	0.71	64.3
	15	0.42	83.3	2.52	90.9	0.17	14.3
30	5	0.17	33.3	4.79	172.7	0.59	50.0
	10	0.92	**183.3**	3.95	142.4	0.59	50.0
	15	0.50	100	2.35	84.8	0.50	42.9
Control	-	0.50	100	2.77	100	1.18	100

According to the obtained results, while administering cobalt compound with dimethylglyoxime and selenocarbamide **(28)** the maximum activity in *Fusarium gibbosum* CNMN FD 12 was recorded at the concentrations of 5-10 mg/L (depending on enzyme types), increase constituting 69.7% for acid proteases and 80.0% for neutral proteases.

In the optimal concentration of 5 mg/L, the marked compound ensures the intensification of neutral protease activity, confirmed by the fact that on the 4-th cultivation day, the proteolytic activity is practically equivalent to that of control variant on the 5-th day – 3.61 u/mL against 3.36 u/mL, respectively.

Table II.21.

The influence of oxime-based coordination compounds of Co(III), Cu(II), Zn(II) on the activity of neutral proteases in *Fusarium gibbosum* CNMN FD 12 micromycete.

Coordination compound	Con. mg/L	The activity of neutral proteases					
		Day 4		Day 5		Day 6	
		u/mL	%, compared to control	u/mL	%, compared to control	u/mL	%, compared to control
28	5	3.61	**286.7**	6.05	180.0	1.26	50.0
	10	1.68	133.3	5.71	170.0	1.26	50.0
	15	1.01	80.0	4.70	140.0	1.05	41.7
29	5	2.02	160.0	6.30	**187.5**	3.53	140.0
	10	1.60	126.7	5.96	177.5	4.20	166.7
	15	1.34	104.3	4.79	142.5	2.52	100
30	5	2.18	173.3	4.66	138.8	3.19	126.7
	10	1.34	106.7	5.88	175.0	3.36	133.3
	15	2.52	**200.0**	6.47	192.5	0.67	26.7
Contr.	-	1.26	100	3.36	100	2.52	100

On the 5-th cultivation day, in all experimental variants, the activity of both types of proteases is superior to the control variant, the activity increase varying between 54.5-69.7% – in the case of acid proteases and 40-80% – in the case of neutral proteases. The maximum activity of acid proteases is ensured by the concentration of 10 mg/L compound, while that of neutral proteases is ensured by the concentration of 5 mg/L.

After administering zinc compound with 1,2-cyclohexanedione dioxime (**30**) in the concentration of 5 mg/L, the activity of acid proteases increased by 72.7%. The superior level of neutral protease activity – 6.17 u/mL – was reached at supplementation with compound in the concentration of 15 mg/L (a 92.5% increase).

At the inclusion of Cu(II) coordination compound with sulphanilamide (**29**) in the nutrient medium, the maximum values of enzymatic activity are reached while administering the minimum tested concentration – 5 mg/L, diminishing concurrently with concentration increase. The activity of acid proteases represents 4.28 u/mL, increase constituting 54.5%. At the concentration of 10-15 mg/L, activity diminishes on average by 5%, under the level of the control variant. Neutral proteases show an activity of 4.79-6.30 u/mL, being by 42.5-87.5% superior to reference variant.

As a result of investigation, it was ascertained the stimulating effect of tested coordination compounds on the activity of acid and neutral proteases in *Fusarium gibbosum* micromycete, the increase constituting 42.4-72.5% for acid proteases and 38.8-92.5% for neutral proteases as compared to control, varying according to applied concentrations.

In all experimental variants, the maximum activity of acid and neutral proteases manifests on the 5-th cultivation day, coinciding with biosynthesis maximum of the control variant. On the 6-th cultivation day the activity of both types of proteases diminishes significantly by 50% and more, as compared to control.

In order to pinpoint the dependence of coordination compounds biological effect on taxon systemacity – genus, species and enzyme systems synthesized in different researches, there were entailed micromycetes from different genera, with different enzymatic systems: *Aspergillus niger* CNMN FD 10 – cellulase and xylanase producer, *Trichoderma koningii* CNMN FD 15 and *Fusarium gibbosum* CNMN FD

12 – protease producer, and from different genera with similar enzymatic systems – *Trichoderma koningii* and *Fusarium gibbosum* – protease producer. Researches were achieved on nutrient media and optimized cultivation regimes background, ensuring optimal conditions both for culture growth and development, and for maximum biosynthesis of enzymes.

As influential factors, there were used oxime based binuclear metal complexes of copper(II), cobalt(III) and zinc(II): $[Zn_2(CH_3COO)_4(NioxH_2)_2bpy(H_2O)_2]$ (**30**) $[Co_2(DH)_4bpeCl_2]\cdot0.5H_2O$ (**31**), $[Cu_2(DH)_4bpe]$ (**32**) [173]. The structural formula of $[Zn_2(CH_3COO)_4(NioxH_2)_2bpy(H_2O)_2]$ (**30**) complex is exposed in Fig. II.18 (p. 97).

In compound **31**, within the central atom, coordinate four oxime group nitrogen atoms of equatorial ligand, and in positions 1,6, there is a chlorine atom and a pyridine nitrogen atom of 1,2-bis(4-pyridyl)ethane ligand that acts as a bridge and joins the complex generator atoms in the binuclear molecule (Fig. II.19).

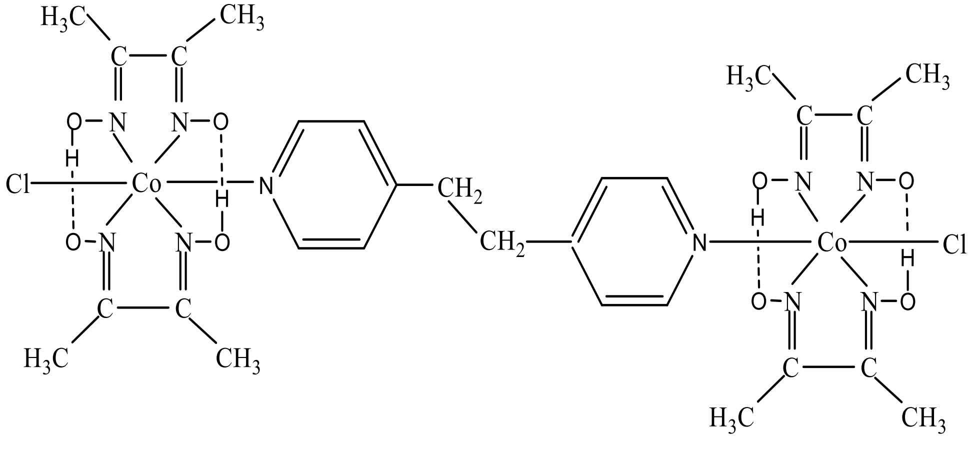


Fig. II.19. The structure of $[Co_2(DH)_4bpeCl_2]$ binuclear molecule.

Within IR spectrum of compound **31** can be found a series of bands at 1627, 1619, 1555, 1511, 1433, 1377, 1237, 1214, 1087, 1035, 976, 868, 813, 739 cm^{-1}. The oxime group is characterized by $\nu(CN)=1555$, $\delta_{as}(N–O)=1237$ and $\delta_s(N–O)=1087$ cm^{-1} bands. The band of region 1619 cm^{-1} can be attributed both to $\nu(CC)$ vibrations, and to $\nu(C=N)$ vibrations, while $\delta(CH)$ band of region 739 cm^{-1}, and the form of bands from this region (the form specific of parasubstituted band ring) are a certain signal of bridging ligand molecules presence in the complex. In region 1511 cm^{-1} can be found the ν_s(C=C) band. The presence of these bands within the OR spectrum of studied compounds confirms the coordination of bpe bridging ligand to the central atom.

Within NMR ^{1}H spectrum of $[Co_2(DH)_4bpe(Cl)_2]\cdot0.5H_2O$ complex, can be noticed a signal in region 2.32 ppm that corresponds to methyl groups of DH ligand. The presence of a single signal proves the fact that methyl groups are magnetically equivalent. The signal of region 18.45 ppm confirms the presence of intramolecular hydrogen bonds within the complex.

The bpe axial ligand manifests in regions 7.33 (2H, d, J=6.18 Hz) ppm and 7.90 (2H, d, J=5.91 Hz) ppm, the corresponding signals belonging to the protons of aromatic rings, and in region 2.79 ppm (4H, s), that belongs to CH_2 groups. The presence of a single signal for these groups also denotes the fact that they are magnetically equivalent. At signals integration within NMR ^{1}H spectrum, It has been found that the ratio between DH and bpe ligands represents 4:1.

In binuclear complex **32**, the complex generator manifests the coordination number 5, in copper ion coordinating for oxime group, nitrogen atoms of the two dimethylglyoxime monoanions connected between them by two intramolecular hydrogen bonds. Pyridine nitrogen of bpe bridging ligand coordinates in position 5 of copper cation (Fig. II.20).

Within IR spectrum of compound **32** can be found a series of bands: 3047, 2916, 1606, 1560, 1501, 1423, 1219, 1073, 1009, 963, 840, 813, 728, 552, 535 and 487 cm^{-1}.

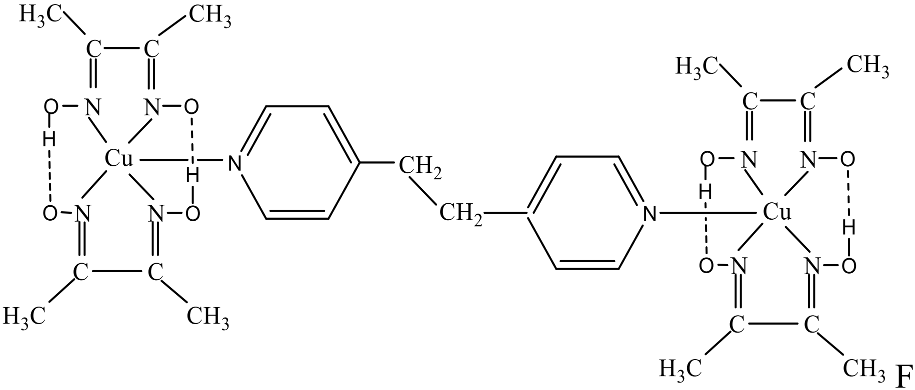


Fig. II.20. The structure of $[Cu_2(DH)_4bpe]$ binuclear molecule.

It has been studied the influence of oxime-based Zn (**30**), Co (**31**), Cu (**32**) coordination compounds on cellulase and xylanase activity in *Aspergillus niger* CNMN FD 10 micromycete.

As a control variant served the micromycete enzymatic activity manifested at cultivation on basic medium (without coordination compounds). The accumulation of cellulases and xylanases in the culture medium was determined in dynamics during 4 cultivation days: 6th; 7th, 8th and 9th day – period where there is a maximum synthesis of enzyme complex components, β-glucosidases endoglucanase (8th), xylanase (7th) under classical conditions of producer cultivation.

After analyzing the obtained data concerning hydrolase activity modification in *Aspergillus niger* micromycete, it has been ascertained that the inclusion of the tested compounds in the producer cultivation medium doesn't influence ß-glucozidase synthesis. Even if on the 6-th cultivation day in experimental variants, cultivated in the presence of the mentioned compounds, records an increase of glucozidase activity – 1.33-1.47 u/mL compared to the level of the day control variant – 1.09 u/mL, on the following cultivation days (8-th and 9-th) the difference attenuates. Glucozidase activity of experimental variants is practically equivalent to control variant activity and constitutes 2.47-2.62 u/mL, against 2.51-2.59 u/mL, respectively. The influence of Co(III) and Zn(II) compounds on endoglucanases is similar: while enzyme activity is superior to control variant on days 6 and 7 – 4.90-5.00 u/mL and 6.36-6.61 u/mL against 4.24 u/mL and 6.12 u/mL in the control variant, it presents lower values on the day of maximum biosynthesis – 7.18-7.34 u/mL against 8.00 u/mL, respectively (Table II.22).

Different from Co(III) and Zn(II) compounds, Cu(II) compound exerts a biostimulating influence on the biosynthesis of endoglucanases. In the compound concentration of 10 mg/L, enzyme activity is superior to control variant during the whole cultivation period, representing 8.81 u/mL against 8.00 u/mL in the control variant on the day of maximum biosynthesis. Additionally, the compound maintains the culture active on the 9-th cultivation day, endogluconase activity constituting 8.16 u/mL against 7.50 u/mL in the reference variant. The extension of stationary phase represents a positive characteristic of the producer as a biotechnological object. In the case of zinc(II) metal complex, the activity of both enzymatic components – ß-glucozidases and endoglucanases – during cultivation days 7-9, remains under the level of control variant in all tested concentrations.

The increase of enzymatic activity at the initial stage (day 6) of cultivation can be attributed to the intensification action of culture development under the influence of coordination compounds, that can reduce microorganism development phases and the precociuos occurance of β-glucozidases and endoglucanases in the cultivation medium.

The tested compounds are of significant interest for stimulating xylanases from the enzymatic complex synthesized by *Aspergillus niger*. On the 6-th cultivation day, it can be noticed a significant increase of xylanase activity in experimental variants – 107.93 u/mL for Co(III) compound, 104.45 u/mL – for Cu(II), 97.48 u/mL – for Zn(II), compared to 94.44 u/mL in the control variant on the 7-th cultivation day – period of maximum synthesis for the control variant. Therefore, the application of the tested compounds accelerates the process of synthesizing xylanases by 24 hours as compared to control, reaching the biosynthesis maximum of the control variant more precociously.

The superior activity of xylanases – 118.81 u/mL on the 7-th cultivation day, was recorded in the case of copper coordination compound, increase constituting 25.8% as compared to control.

Cobalt and copper binuclear compounds can be considered veracious stimulators of xylanase biosynthesis in the strain of *Aspergillus niger* CNMN FD 10, ensuring the increase of enzyme biosynthesis in all variants of tested concentrations, and the extension of stationary phase, by maintaining the culture active during 72 hours. Zinc metal complex with 1,2-cyclohexanedione dioxime and 4,4-bipyridyl don't enhance xylanase biosynthesis, enzyme activity (94.0-97.48 u/mL) remains at the level of control variant on the day of maximum biosynthesis for culture, but stimulates the precocious manifestation of the maximum by 24 hours, fact presenting technological efficiency at obtaining enzymatic preparations.

Table II.22.

The influence of oxime-based coordination compounds of zinc, cobalt and copper on cellulase and xylanase activity of *Aspergillus niger* CNMN FD 10 micromycete.

Coord. comp.	Conc. mg/L	β-glucozidases				endoglucanases				Xylanases			
		6 day	7 day	8 day	9 day	6 day	7 day	8 day	9 day	6 day	7 day	8 day	9 day
30	5	1.33	2.47	2.28	1.90	5.00	6.61	7.34	6.04	94.00	76.38	87.04	71.37
	10	1.25	2.37	2.44	1.95	4.90	6.61	3.59	6.36	**97.48**	84.86	91.39	73.11
	15	1.20	2.28	2.59	1.69	5.00	6.04	3.26	6.36	94.21	90.52	91.39	87.04
31	5	1.28	2.28	2.44	1.85	4.95	6.36	7.34	6.20	**107.93**	89.11	105.53	60.93
	10	1.47	2.28	2.47	1.82	5.00	6.53	7.18	6.04	100.97	**104.66**	104.45	64.41
	15	1.28	2.21	2.28	1.82	3.59	5.55	6.53	5.87	97.48	82.03	87.04	62.66
32	5	1.25	2.48	2.55	1.90	4.79	6.12	7.99	8.16	**104.45**	107.49	113.52	86.17
	10	1.31	2.17	2.62	1.66	4.90	6.53	**8.81**	8.16	104.45	**118.81**	91.39	90.52
	15	1.36	2.10	2.40	1.48	0.14	5.71	7.18	6.69	104.45	118.81	91.39	64.41
Control	-	1.09	2.51	2.59	1.90	4.24	6.12	8.00	7.50	79.21	94.44	80.51	63.54

Research results prove the selective influence of the tested coordination compounds on the biosynthesis of enzymatic components of cellulase-xylanase complex synthesized by *Aspergillus niger* CNMN FD 10 micromycete: the introduction of the tested compounds in the mycromycete cultivation medium doesn’t influence the biosynthesis of β-glucozidases but influences differentiatedly the biosynthesis of endoglucanases and xylanases. The results correspond to preliminary data obtained in similar researches with coordination compounds, different from the compounds used in recent researches on cellulase producer *Penicillium expansum* CMNM FD 04 strain. Co(III) and Zn(II) compounds modify the term of maximum manifestation of xylanase biosynthesis from day 7 to day 6, reducing the micromycete cultivation cycle by 24 hours. Cu(II) metal complex increases the activity of xylanases as compared to the reference variant, ensuring a rise of 25.8%. The mentioned effects ensure the possibility to connect the composition of enzymatic preparations in relation to the exigencies of application sphere and technological efficiency in enzyme production.

In order to elucidate the action mechanism of metal complexes on the capacity of producer strain, at the subsequent stage, it was studied the influence of coordination compounds components. The components of metal complexes were added to the cultivation medium in concentrations equivalent to their concentration within the compound [103, 132-134].

According to data exposed in tables II.23 and II.24, it should be underlined the complex beneficial effect and not that of ligands or metal complex components taken separately.

Table II.23.

Modification of amylolytic activity on the 5-th cultivation day of *Aspergillus niger* 33-19 strain in the presence of $[Co(DH)_2(An)_2][TiF_6]\cdot 2H_2O$ coordination compound and its components in the optimal concentration of 10 mg/L.

Substance	Amylolytic activity			
	pH 4.7		pH 2.5	
	u/mL	% / C6	u/mL	% / C6
2An	98.71	46.46	109.24	37.97
2DH	114.92	54.09	128.94	44.81
$CoTiF_6\cdot 6H_2O$	188.25	88.61	201.00	69.86
$[Co(DH)_2(An)_2][TiF_6]\cdot 2H_2O$	272.20	**128.12**	349.63	**121.52**
Control 5	190.35		247.7	
Control 6	212.45		287.75	

Table II.24.

Modification of amylolytic activity, on the 5-th cultivation day, of *Aspergillus niger* 33-19 strain in the presence of $[Co(NioxH)_2(Sam)_2][TiF_6]\cdot 3H_2O$ coordination compound and its components in the optimal concentration of 5 mg/L.

Substance	amylolytic activity			
	pH 4.7		pH 2.5	
	u/mL	% / C6	u/mL	% / C6
$2NioxH_2$	226.81	106.76	244.09	84.84
2Sam	215.30	101.34	228.73	79.50
$CoTiF_6\cdot 6H_2O+CoCl_2\cdot 6H_2O$	182.46	85.88	218.52	75.95
$[Co(NioxH)_2(Sam)_2][TiF_6]\cdot 3H_2O$	303.40	**142.81**	376.51	**130.87**
Control 5	190.35		247.7	
Control 6	212.45		287.75	

Conclusions:

Multilateral performances of fungi as producers of bioactive substances and agents for creating eco-friendly technologies underline mycelia fungi as main producers of biotechnologies, able to substitute plants, animals and bacteria, fact simplifying and increasing the efficiency of technological processes.

Results are obtained due to the reduction of biological objects development period, the use of cheap cultivation media, the possibility to create polyvalent biotechnologies with a closed cycle and reduced ecological risk. It is assumed that there are about 1.5 mln species of fungi on Earth. Taking into account the fact that until now all contributions involving fungi were achieved with the implication of just 5% of the total species existing in nature, fungi represent a promising genetic potential for biotechnologies.

Fundamental achievements in mycology carried out in the last two decades increased scientists' interest for mycelia fungi as potential producers of progressive biotechnologies for medicine and pharmaceutical industry. The development of oriented synthesis technologies for enzyme preparations production with selected fungi strains as sources of enzymes, the establishment of qualitative and quantitative ways of controlling their biosynthetic potential, the creation of economically advantageous and ecologically harmless proceedings for regulating and controlling microbial processes are actual, pertinent and correspond to the world trends of society development.

The use of coordination compounds of transition metals in the biotechnology of mycelia fungi cultivation as sources of bioactive substances is a direction with a high potential. The promising results obtained in the current researches and field, open huge, fundamental and innovative opportunities.

As a result of investigation, there have been developed optimal conditions for applying cobalt(III) dioximates with fluorine in the process of enhancing the biosynthesis of standard and acid stable amylases in *Aspergillus niger* 33-19 CNMN FD 02A strain of exocellular lipases in *Rhizopus arrhizus* Fischer CNMN FD 03L strain.

The compound of cobalt(III) dioxime with sulphanilamides – $[Co(NioxH)_2(Sam)_2]_2[TiF_6]\cdot 3H_2O$, in the experiments with *Aspergillus niger* 33-19 strain, manifests as a real stimulator of amylase biosynthesis.

In all tested concentrations, it was recorded an increased activity of both types of amylases – acid-labile and acid-stable, respectively by 40.47-164.08% and 33.9-148.15%.

The inclusion of $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot3H_2O$, $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot2H_2O$, $[Co(DH)_2(Nia)_2][BF_4]\cdot2H_2O$ coordination compounds containing complex anions with zirconium, boron and fluorine in the concentration of 1-5 mg/L, modified the cultivation duration of *Aspergillus niger* 33-19 CNMN FD 02A strain, reducing the technological cycle by 24 hours, the enzymatic activity of experimental variants being superior to control (5.5-11.1%) on the first cultivation day.

The introduction in the cultivation medium of $[Co(DH)_2(An)_2]_2[TiF_6]\cdot2H_2O$ coordination compound containing the complex anion with titanium and fluorine, in the concentration 5-10 mg/L, reduces the technological cycle by 48 hours, ensuring the increase of acid stable amylase activity by 27.8% and that of standard amylases by 23.69%. Enzymatic activity in this variant remains superior to control variant during the whole cultivation period.

The tested cobalt(III) dioximates with fluorine do not modify the duration of *Rhizopus arrhizus* Fischer CNMN FD 03L micromycete cultivation – exocellular lipase producer.

At introduction of $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot3H_2O$ and $[Co(DH)_2(An)_2]_2[TiF_6]\cdot2H_2O$ compounds in the cultivation medium of *Rhizopus arrhizus* Fischer CNMN FD 03L micromycete, it was recorded a superior lypolytic activity in the control samples, namely in the variants with the concentration of 1 mg/L for both complexes, constituting 68750 u/mL and 75000 u/mL respectively, compared to 56250 u/mL on the control medium, fact representing an increase of 22.2% and 33.3%. The highest pectolytic activity was recorded in the variants with $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot2H_2O$, $[Co(DH)_2(Nia)_2][BF_4]\cdot2H_2O$ coordination compounds, constituting, at the application of the concentration of 5 mg/L, 93750 u/mL and 87500 u/mL respectively, as compared to 56250 u/mL in the control medium, the increase representing 66.6% and 55.5%.

It was recorded the influence of the external sphere of the tested cobalt(III) coordination compounds with fluorine on the processes of enzymatic development and synthesis in the mycelia fungi producing exocellular hydrolases.

On the example of micromycete strains of *Aspergillus niger* CNMN FD 10 – producer of cellulases and xylanases, *Trichoderma koningii* Oudemans CNMN FD 15*, Fusarium gibbosum* CNMN FD 12 – producers of acid and neutral proteases, it was proved the dependence of the effect exerted by Co(III), Cu(II), Zn(II) coordination compounds with oxime ligands upon the biosynthesis of exocellular hydrolases on the taxonomic position of micromycete strains and on the synthesized enzymatic system.

It was established the differentiated effect of the tested compounds on the components activity of the cellulase and xylanase complex synthesized by the strain and on the acid and neutral proteases in *Trichoderma koningii* Oudemans CNMN FD 15 strain.

The introduction of the tested compounds in the cultivation medium of *Aspergillus niger* CNMN FD 10 micromycete doesn't influence β-glucosidase biosynthesis but, differentiatedly, influences xylanase biosynthesis. Thus, Co and Cu compounds ensure the increase of enzyme biosynthesis in all variants of tested concentrations and the stationary phase extension, maintaining the culture active about 72 hours. The metal complex of zinc doesn't increase xylanase biosynthesis, the enzyme activity (94.0-97.48 u/mL) remains at the level of control on the day of culture maximum biosynthesis, but it accelerates the precaucious manifestation of the maximum by 24 hours (from day 7 to day 6).

Cobalt compound with dimethylglyoxime and selena carbamide $[Co(DH)_2(Seu)_{7/4}(Se\text{-}Seu)_{1/4}]_2[TiF_6]\cdot H_2O$ – increases the biosynthesis of both types of proteases of *Trichoderma koningi micromycete*: acid – by 37.1-122.0%, neutral – by 13.8-44.1%. The complex doesn't modify the vital cycle of strain development.

Copper coordination compound with di-sulphanilamide glyoxime increases the biosynthesis of acid proteases by 110.7-121.4% against control by accelerating the maximum manifestation from day 9 (in the control variant) to day 8. The maximum of neutral protease biosynthesis manifests as in the control variant – on the 9-th cultivation day, exceeding the level of control activity by 52.5%

Zinc compound manifests an obvious stimulating effect on the activity of acid and neutral proteases of *Trichoderma koningii* micromycete, by accelerating the manifestation of biosynthesis maximum for both types of proteases from day 9 (control variant) to the 8-th cultivation day, the maximumincrease for acid proteases constituting 110.2%, for neutral proteases – 69.7%, being ensured by 5 mg/L of compound concentration.

It was ascertained the similar influence of the tested compounds on protease biosynthesis in *Fusarium gibbosum* CNMN FD 12 strain, ensuring the increase of acid proteases by about 49-73% and of neutral proteases – by 80-93%, the maximum activity of both types of protease being recorded on the 5-th cultivation day, analogous to the control variant.

The study of the influence of individual components on the strain producing capacity has demonstrated the beneficial effect of the complex as a whole, not of the ligands or metal complex components.

The above mentioned effects ensure the efficiency of enzyme production technology and the possibility to connect the composition of enzymatic preparations to the exigencies of the application domain.

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Chapter III.

THE EFFECT OF SOME COORDINATION COMPOUNDS ON PLANT PHYSIOLOGICAL PROCESSES UNDER THE IMPACT OF ECOLOGICAL STRESS

III.1. Introduction. Research originality

Increasing plant productivity and food security in conditions of external environment unfavorable fluctuations, particularly of climatic factors provenance, continues to be one of the most pressing problems of modern agriculture. Considering the increase of greenhouse effect, air temperature and the frequency of dry weather, it is often raised the problem of improving major crops resistance to drought. Water scarcity accessible to the plant causes partial or total closure of stomata and, therefore stops photosynthesis. The resultant reaction obstructs growth, biomass accumulation and plant productivity. For agricultural countries located in the semi-arid steppe zone (like R.M.), the development and practical implementation of tolerance inducing methods, directed towards reducing impact and stabilizing productivity, opens big prospects.

Traditionally, the mitigation of drought impact can be ensured by irrigation, breeding tolerant plants, zoning in accordance with requirements and genotype resistence, compliance with agro-technical measures, etc. An important opportunity to increase crop productivity, not valuated yet, is considered the use of physiologically active substances (PhAS), able to regulate growth and development, modify various metabolic processes, and intensify the adaptive properties of the vegetal organism in environmental suboptimal conditions. Currently, worldwide, attention is given to compounds with a broad-spectrum activity, that combine properties of physiologically active substances, possess trophic effects, are active at low concentrations, and can increase productivity and yield quality. Among the latest PhAS, attention should be given to the complex compounds with trace metals, derivatives of natural compounds, contained in plants. Through numerous investigations, it has been established that they influence plant adaptive properties, fact enabling reduction / dosage of used chemicals and those of hormone type, and pressing on environment. The importance of these bioactive compounds for agriculture is obvious on grounds of increasing work rentability.

In fact, all physiological and agronomic researches have as a final objective the elucidation of laws and complex mechanisms of plant growth and development, so that on their basis could be created the most favourable conditions to fully achieve plant productivity potential. Since a certain period of time, in science it was created a correct general conception about the fact that the growth and development program of each plant species and variety is codified in its genetic apparatus, and this program fulfils in accordance with environmental conditions, growth and development modifications within the limits of plant adaptive capacities, in other words within the limits of body reaction norm. Water shortage in the soil inhibits plant growth, its primary and agronomic valuable productivity being linearly dependent on the saturation deficit of organs. Plants directly perceive humidity and conservatively react to water potential decrease in the soil – faster than it diminishes the capacity of roots to extract water to meet the needs of leaves. Regretfully, there is a conflict between the mechanisms that induce plant resistance and adaptation to drought and those ensuring their high productivity [1].

The need to explore new physiologically active substances that could be used in agriculture stems from the fact that unfavorable environmental conditions, especially extreme fluctuations in temperature and humidity, have a negative influence on plant metabolic processes, growth, development and productivity, with sub-lethal or lethal consequences. Although agricultural production in the last 40-50 years increased 2.5 times, due to genetic and technological improvements, a further increase is unlikely in conditions of global warming, diminuation of precipitations quantity and increase of CO_2 and other gases concentration. The effective and nontoxic PhAS perspective becomes particularly timely at this stage of national economy development on grounds of ecology issues and the need to increase crop productivity. Even Nikel J. (1984) mentioned that the prospect of using PhAS will always be growing because of higher energy prices, reduced sowing areas and the need to increase food production.

Plant resistance to humidity deficiency consists of a complex of protective mechanisms – physiological, biochemical, anatomical and morphological – aimed at maintaining internal aqueous environment at a relatively constant level, a prerequisite for normally attending anabolic and catabolic processes. During evolution, in plants formed various adaptive features that facilitate their survival in arid or subaride conditions. Plant adaptive capacity is fixed in a genetic program, whose deployment is coordinated in time and depends on specific conditions of external environment [2]. Mediators between genetic and environmental programs are phytohormones – a premise to regulate exogenous vital processes and body tolerance to unfavorable conditions [3-5]. Based on these statements, it was formulated the hypothesis that PhAS with the effect of optimizing water status in plant will have a positive impact on plant productivity and resistance under moderate humidity deficiency. So, drought can be mitigated by using bioactive substances with an effect of attracting water in the most important organs for survival and yield formation.

Another cause for disrupting and damaging vital processes in plants with a haywire hydric regime can be the diminuation of nutrients accessibility for plants. It is known that in dry conditions mineral elements of the soil become inaccessible. Insufficient content of some elements in assimilable form, the presence of heavy metals in mobile form worsen crop growth and development, and significantly reduce yield. On the other hand, soil drying in the root system zone causes the destruction of absorbent hairs and the inhibition of roots uptake activity, thus increasing the deficiency of water and nutrients in tissues. Micronutrients scarcity or inaccessibility leads to their discrepancy in organs, followed by serious disturbances in the functional activity of the organism, which manifests through various functional disorders: illnesses or plant organs drying. The problem of negative complex effects of drought, as well as humidity excess, in the context of energy resources and raw materials crisis in the world, urged the search for new sources of fertilizers with trace elements such as waste and the creation of economical technologies for their use in plant cultivation [6-8].

In recent years, due to the widespread use in agriculture, medicine and animal husbandry, an increasing interest has been observed towards coordination compounds with various metals, that possess properties of PhAS [9-12]. The interest in this class of substances can be explained by their high efficiency as anti-chlorotic concoctions, by their capacity to activate multiple biochemical reactions, to easily penetrate and carry important cations by means of plant and animal cell membranes, etc. [13-15]. Besides, complex compounds with bioactive and transition metals display a superoxiddismutasic activity, property of inhibiting microsomal processes [16, 17], that activates in unfavorable conditions and represents the primary cause of cell distruction. It was mentioned the significant perspective of these substances to increase plant productivity and resistance to adverse environmental conditions and diseases, and to improve yield quality [18-23]. Bioactive complex compounds of metals, as opposed to mineral fertilizers, can be used at much lower concentrations. Ostrovskaia L. and co. (1984, 1986) mentioned [17, 22], that PhAS use is economically and effectively entitled to administration by means of any method. There are data that demonstrate the beneficial effect of PhAS on plants functional status and yield, even if plants don't obviously need that or another microelement [12, 24, 25]. In investigations conducted on the basis of financial contracts and collaboration with scientists from Russia, it had been established the property of bioactive complex compounds, derivatives of succinic acid with various cations (Fe^{3+}, Co^{2+}, Zn^{2+}, Mg^{2+} etc.), to stabilize metabolic reactions coupled with energy generation and consumption of various plants (apple, corn, potato) under humidity favorable and unfavorable conditions [12, 26].

On the other hand, trophic factors regulating growth everywhere and for a long time additionally include vitamins of group B [27-31]. Vitamins are considered the most innocuous substances among the organic nutritional factors and are necessary for the continuous growth of all organisms. Partly, vitamins (B_1, B_6, PP, C) are directly involved in root and whole plant metabolic processes and in those related to the absorption of substances from the soil, the formation of root system new structures, others can be eliminated in the soil, thus forming rhizosphere environment [31]. In autotrophic organisms, despite the fact that vitamins are endogenously synthesized, their exogenous application exercises the most positive effect on CO_2 uptake, protein synthesis and plant growth. It was established the peculiarity of ascorbic acid to protect the photosynthetic apparatus from destructive effects [32], thiamine capacity to increase chlorophyll resistence and, therefore, to foster growth and productivity. However, there are insufficient works regarding vitamins exogenous supplement effects on the process of growth, development and productivity of crop plants.

A way of increasing plant tolerance to drought and heat, aimed at directing certain components of water status, is the use of certain „protector", „antistress" or „antiperspirant" substances. There were found to be very effective and promising the aqueous emulsions of some synthetic macromolecular compounds which form a monomolecular film on the surface of leaves without interfering with photosynthesis process.

The review of information about PhAS influence with antiperspirant effect on plant functional processes and productivity had been exposed in the below mentioned works [33-42]. During plant growth and development, the regulation of stomatal aperture permanently occurs with natural antitranspirant involvement – epicuticular wax that influences diffusion of water vapours and carbon dioxide [43]. It had been demonstrated the beneficial effect of such films as hexadecane, polyethylene, polypropylene, polystyrene, polyvinyl chloride, latex, natural and synthetic, latex that form films on leaves without influencing biochemical processes. At the same time they rather limit water vapours denudation from mesophile in atmosphere than carbon dioxide penetration in leaves and do not cause negative changes in photosynthesis, respiration and cell thermal regime [44]. A beneficial influence on reducing transpiration intensity have the humic and acetylsalicylic acids. Antitranspirants can provide the reduction of water evaporation by 30 percent as compared to control [35, 45-50]. As mentioned in numerous scientific papers, physiologically active antitranspirants are widely applied in countries with an arid climate for regulating fluid balance in tissues and reducing yield loss under stress conditions.

Due to its unique physical and chemical properties (solubility in water and organic solvents, good adhesion to organic and inorganic substrates, complexing with various reagents tendency, lack of toxicity, etc.) poly-N-vinylpyrrolidone is used in various fields [51]. In medicine, depending on the molecular weight, polyvinylpyrrolidone is used [51]:

- to solubilize some medicines and eliminate toxic substances from the human body (M ~ 10000-15000);
- to substitute plasma during surgery (M-25000-40000);
- to prolong the biological activity of drugs (M-60000).

Polyvinylpyrrolidone is characterized as a prolongator, prolonging the action of drugs in the human body. Polyvinylpyrrolidone, cetylpyridinium hydrochloride, sodium citrate, sorbitol, metoprolol and other ingredients are used to obtain bactericid, antifungal films and water-soluble films used to pack drugs [52-54]. The number of works connected to the use N-vinylpyrrolidone, polymers and copolymers in village household is still small but growing [51, 55, 56]. N-vinylpyrrolidone compounds are used in veterinary, animal husbandry and agriculture to prolong the action of medicine, pesticides, and microelements, to solubilize certain poorly water-soluble preparations. Polyvinylpyrrolidone serves as a component of fertilizers, fodder for livestock and poultry (raises food consumption efficiency). Its complex with iodine protects plants from insects, fungi and nematodes [51]. It was shown that the aqueous polyvinylpyrrolidone solution of 5-20% reduces water loss from plant cells [55].

It had been established [57] that the use of PVP compositions and other polymers with bioactive preparations like fentiuram as mordars to process seeds generate the double reduction of bioactive preparations and the increase of cotton yield and other crops by 10-15%. R. Muhamedova demonstrated that bioactive preparations grafted on polyvinyl alcohol increase plant resistance to various pathogens and, consequently, lead to the increase of corn, potatoes and sugar beet yield.

Thus, the analysis of information on the problem indicates that rapid population growth, expansion of advanced technology development, environment rapid degradation, and as a result of this situation, higher requirements for life quality, set new goals to science – explore ways and means that would ensure stable development of the country's economy or would overcome the ecological crisis. However, the emphasis is on ecological and food security in all spheres of activity. Through ecological safety it should be understood the protection of people and ecosystems against the negative consequences of natural and technogenic disasters, and of anthropogenic activities on the environment [58]. In this context, the exploration of new ways of tempering drought impact in the agrarian sector of economy takes on a new hue and must be analyzed in terms of protecting the environment and human health. Another objective of the study was to broaden the assortment of physiologically active substances with an ecologically significant effect on plant productivity and yield quality and develop methods of using them in order to express potential resistance to drought. Until recently it hasn't been clarified the practical use of biologically active coordination compounds in increasing crop plants tolerance to adverse environmental conditions. Therefore, it is important to study the effect of a series of coordination compounds, synthesized at the Institute of Chemistry of the ASM, on physiological processes, growth, development and productivity of crop plants under adverse temperature and humidity. There had beed conducted researches in order to elucidate the properties of coordination compounds to increase the activity of antioxidant protection enzymes or decrease the formation of reactive oxygen species in plant cells exposed to adverse conditions of external environment. The researches had been conducted in laboratory experiments, within a Vegetation Complex and on the fields of the Institute of Genetics, Physiology and Plant Protection of the Academy of Sciences, on small plots, as required by the State Center for testing and approving fertilizing and phyto amendatory products.

The dioximates of transition metals are part of a branch of chemicals situated at the intersection of inorganic and organic chemistry

– coordinative chemistry. This includes the most various by structure and properties substances, many of which shape the active centers of some important natural compounds such as hemoglobin, chlorophyll, insulin, B_{12}, cytochromes et al., showing useful properties for the vital activity of normal living organisms. The composition and structure of complexes play a key role in the manifestation of properties, including biological activity, therefore, dioximates study was carried out in parallel with complexes of another class of coordination compounds – homo- and hetero-trinuclear clusters of μ_3-oxo type.

III.2. Study objects, research methods

The research had as study objects the plants of *Zea mays L.*, M 291 and P 459 hybrids; *Phaseolus vulgaris,* L., Porumbiţa variety; *Glycine max* Merr. (L.) Bucuria variety; *Cucumis sativus* L., Concurent and Mirabella varieties and the hybrids of Rodnichoc F_1 and Icar; *Lycopersicon esculentum* L. cv. Lider; *Arachis hypogeae (L.),* Fazenda variety 2; beet plants *(Béta vulgáris* L. var. conditiva Alef.); sugar beet *(Béta vulgáris* L., Baracuda variety), garlic (*Allium sativum* L. Izumrud variety) etc.

It is known that the most objective criterion for assessing the influence of PhAS is biomass and the agriculturally valuable part of the crop. Therefore, there had been carried out laboratory experiments, during which it was tested PhAS action on growth processes at early stages of development, experiences of vegetation, under controlled conditions of humidity, and field experiences, that offer the opportunity to test PhAS effect on plant productivity.

During laboratory experiments it had been studied the effect of seed treatment by means of vacuum-infiltration and incrustation with different PhAS doses on start reactions of ontogenesis. It had been checked imbibition rate, mobilization of reserve assimilates, germination energy, biomass accumulation and planlets growth at early stages of ontogenesis, in order to establish the PhAS dose with an optimal effect on physiological processes. At the initial stages of ontogenesis, there had been performed researches on planlets of 7-8 days after germination, grown out of seeds infiltrated with water or appropriate solutions of PhAS of various concentrations. Seeds germination took place in rolls of filter paper in glass, with appropriate solution or water, built in thermostats at 27° C and 70% humidity. During experiments, there had been determined, per organs, germination energy, linear growth parameters – rootlet, adventitious roots and coleoptile length, the number of adventitious roots and seedling biomass.

In field experiments, the seeds had been encrusted with PhAS before sowing and had been treated foliarly with the appropriate solution at various stages of development. The experiments had been carried out by blocks, in three replicates, with randomly arranged variants. About the PhAS effect on corn plants, it was concluded by identifying the differences of growth processes morphophysiological parameters, primary productivity and plant agriculturally valuable part. The estimation of adaptive reactions was performed by determining height, leaf area, biomass, productivity and yield structure. Morphogenetic indices were determined as recommended. Leaf area, as average of all leaves, was calculated by the equation: $S=L\cdot l\cdot 0.65$, where L – length, l – leaf width; the specific weight being calculated as leaf weight relationship relative to surface. This parameter characterizes the economic consumption of assimilates in the process of forming foliage. In order to determine the peculiarities of water status during the study, there have been established the indices below and used the following approved methods: water content, saturation deficit, capacity of water retention in tissues, perspiration intensity, hydric potential, osmotic potential, hydraulic conductivity in the „root-shoot" segment, linear growth and biomass accumulation, water use efficacy. The total water content in leaves, stems and roots was determined by drying the sample to a constant weight at 105° C in a drying cupboard with temperature control of 2B-151 type. Saturation deficit in leaves is expressed in percent of full saturation. The ability to retain water in tissues characterized by water loss over a period of time expressed in percentage towards the original content, as well as by the amount of retained water. In this study it was applied the determination of hydric status in one and the same sample. All the data on the parameters of water status in bodies were shown as the mean of 5 reproductions ± standard error, and as the average of change degree in 3-5 experiments. Measurements were performed yearly in the morning, when the plants are the most sensitive to drought.

Water potential (Ψ_w) was determined throughout the compensation method [61]. Turgor potential (Ψ_p) was obtained from water potential and osmotic difference: $\Psi_p=\Psi_\pi-\Psi_w$. Cellular juice concentration was measured by means of Larcher Carat refractometer type [60]. Daytime perspiration intensity of different leaves that differed by age was performed using A. Cornescu transpirometers [61]. In laboratory experiments, hydraulic conductivity was determined by inhibiting water channels with 0.5 mM solution of $HgCl_2$, as recommended [62, 63], and calculated, taking into consideration that the driving force of water from roots is based on osmotic pressure gradient in the segment of „root-leaf" – $L_p=F_v/\Delta\Psi_\pi$ where L_p – hydraulic conductivity (mg·h^{-1}·g m. p., MPa^{-1}), F_v – water flow rate, $\Delta\Psi_\pi$ – osmotic pressure difference of root and leaves juice [64]. Hydraulic conductivity of plants was calculated as a result of the diurnal relationship between the water potential of leaves and stems and juice stream or perspiration rate: $F=L_p(\Psi_{w\ leaf}-\Psi_{w\ trunk})$, where F – transpiration rate, or water flow; L_p – proportionality factor, defined as plant hydraulic conductance [65-67].

Given the fact that water flow through plant is maintained thanks to the gradient of water potential between soil and transpiring leaves area, perspiration rate was considered as a value of the latter. About water use efficacy, it was concluded on grounds of the relation between consumed water and living matter production [68]. The evidence of morphogenetic peculiarities and plant productivity was statistically analyzed using „Statistics 6" software package for computers.

At the basis of research methodology lay the principle of estimating PhAS efficacy and ascertaining their action manifestation on physiological or biochemical processes. Testing PhAS effectiveness on alternative reactions of metabolism opens the prospect for selecting the most economically profitable, for ecological farming, elaborations with a certain orientation [69]. The author brings as example the bioactive coordination compounds with metal-microelements, whose norms of use are significantly smaller than the administration norm of the same metal-microelements in the form of inorganic salts. Low doses of PhAS may exhibit pronounced bioactive effect only if they act on plant regulating systems or when they chemically interact with plant cell substrates [70].

III.3. Results and discussions

The influence of coordination compounds on the growth and productivity of Zea mays L. plants.

It is considered that seed treatment is one of the most technological proceedings of using microelements. The main priorities are: low consumption of micro fertilizers, low environmental pollution and provision of microelements action on the early formation of metabolic systems. Microelement biological activity increases while using metal in the form of hydrosoluble complexones.

There were carried out a series of studies concerning the influence of some chemical compounds of different classes (carboxylates, dioximates, compositions formed of compounds containing micro and / or macro-elements, vitamins, biologically active substances, etc.) on certain physiological parameters determining qualitatively and quantitatively seed germination energy, the content of assimilating pigments, carotinoids, malondialdehyde etc., and on the productivity of some crop plants and their production quality.

The study of iron(III) carboxilates began in 1906-1926, the base being laid by Weinland R. and Gussman E. [71]. Yet, the existing physic-chemical data, at that moment, were not sufficient to make adequate conclusions regarding their molecular structure.

The first molecular structure of this type of complexes was desciphered on the example of $[Fe_3O(CH_3COO)_6(H_2O)_3]ClO_4$ [72], and later on the example of $[Fe_3O(CH_3COO)_6(H_2O)_3]NO_3 \cdot 4H_2O$ [73] (Fig. III.1). The structure contains three iron atoms, forming a nearly isosceles triangle, joined by an oxygen atom, as a bridging ligand in the center of the triangle, each by two anions of carboxylic acid bidentatedly coordinated.

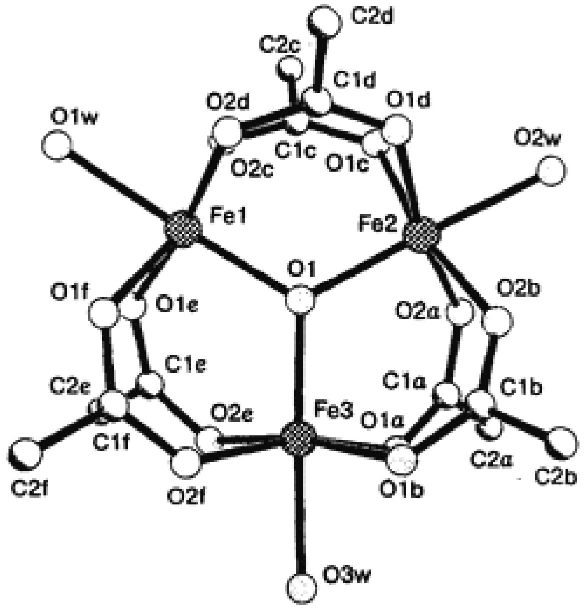

Fig. III.1. The structure of the complex cation within $[Fe_3O(CH_3COO)_6(H_2O)_3]NO_3\cdot 4H_2O$.

Each iron atom forms an octahedron, wherein the sixth coordinate is occupied by a molecule of water [73] or other monodentate neutral ligand [74]. There were obtained complexes with fatty carboxylic acids of $[Fe_3O(C_{5\text{-}7\text{-}9}H_{11\text{-}17\text{-}19}COOH)_6(H_2O)_3]NO_3(Cl)$ [75], and $[Fe_3O(CH_3COO)_6(H_2O)_3]NO_3\cdot 4H_2O$ [76], subsequently studied by using IR, electronic and Mössbauer spectroscopy, magnetochemistry, complex thermal analysis and quantum chemistry. Within them, take place antiferromagnetic interactions specific of μ_3-oxo-carboxylate clusters. The calculated theoretical values of (DI_{Na^+}) isomer shift and quadrupole splitting (QD) correspond to experimental values [73]. Taking into account theoretical calculations, researchers [73] assumed that the antiferromagnetic interaction between Fe(III) ions characteristic of $[Fe_3O(RCOO)_6(L)_3]^+$ cations takes place mostly by means of μ_3-oxo bridging.

Homotrinuclear clusters of μ_3-oxo-carboxylates type, with the general formula $[Fe^{III}{}_3O(RCOO)_6L_3]X$ ($R=CH_3$, L= nicotinamide (vitamin PP), N,N-diethylnicotinamide, ethyl ester of nicotinic and isonicotinic acids etc.) are well known and studied in details [77]. Homonuclear clusters of μ_3-oxo-carboxylates type form, thereto, other transitional metals: vanadium [78], chromium [79], manganese [80, 81], in some compounds the metals being mixed-valent [82], and ruthenium [83]. Synthesis, magnetism and Mössbauer spectra of trinuclear compounds $[Fe_3^{III}O(CH_3COO)_6(H_2O)_{3-n}L_n]X\cdot m(Solv)$ ($X=NO_3^-$; L= 3-$CON(C_2H_5)_2$-Py, 3- or 4-$COOC_2H_5$-Py; Solv =H_2O or CH_3COOH of μ_3-oxo clysters type are exposed in [77]. The iron is in +3 oxidation state and in high spin state (S=5/2).

The value of the effective magnetic moment for an iron atom at room temperature is equal to $\approx 3\mu_B$, lower than the theoretical one (5.9 μ_B), while at 4.2 K – it is equal to 1.5 μ_B, fact indicating on the presence of antiferromagnetic interaction between Fe paramagnetic centers, that, as researchers presume [73], takes place by means of the central oxygen (μ_3-oxo). An antiferromagnetic interaction was also observed between Cr(III) ions within the complex of $[Cr_3(O_2CCH_3)(na)_3]PF_6\cdot 3CH_3CN$ [79].

The results of magnetic measurements of $[Fe_3^{III}O(CH_3COO)_6(Dena)(H_2O)_2]$ and $[Fe_3^{III}O(CH_3COO)_6(Dena)_2(H_2O)]$ show [77] that the dependence of magnetic susceptibility on temperature doesn't subdue to Curie-Weiss law. The values of magnetic moments relative to an iron atom at room temperature are equal approximately to 3 μ_β, values essentially lower than the pure one for Fe(III) high S=5/2 (5.9 μ_β). At 4.2 K the value of the effective magnetic moment relative to an iron atom decreases to 1.5 μ_β. Such a behavior of $\mu_{eff.}$ can be explained through the presence of antiferromagnetic interaction among iron paramagnetic ions [77] within the respective compounds.

Researchers [73] interpreted the IR spectrum of $[Fe_3O(CH_3COO)_6(H_2O)_3]NO_3\cdot 4H_2O$, whose structure is characterized as being of „carcass" type. Hence, the acetate anion is attributed the following absorption bands, cm^{-1}: $\nu_{as}(COO)$ = 1595 and $\nu_s(COO)$ = 1450 with $\Delta\nu$ =145, $\delta_{as}(OCO)$=660, $\pi(COO)$ = 614, and $\rho(COO)$ =530; $\nu(CH)_{(CH_3)}$=2930 and 2860, $\delta(CH_3)$=1430 and 1380. Water molecules absorb, in region 3600-3200 cm^{-1}, the group of NO_3^- – at 972 and 828 cm^{-1}. The oscillations of $\nu_{as}(Fe_3O)$ manifest in region 595-602 cm^{-1}, while $\nu(FeO_4)$ – at 363 cm^{-1}.

The results of the study of the kinetics and mechanisms of electron transfer reactions from homonuclear compounds of $[Fe_3(O_2CR)_6L_3]ClO_4$ with verdazyl radicals (R=CH_2CN, CH_2F, CH_2Cl, CH_2Br, p-$NO_2C_6H_4$; L=Py, 3-, 4-CH_3-Py, 3,5-$(CH_3)_2$-Py, 3-CN-Py, 3-F-Py), by means of cyclic voltammetry method, are reflected in [74].

In addition to homonuclear complexes, the metals also form heteronuclear compounds that, unlike the first, contain different metals, in most cases of *d* and *s* types. Formally, heteronuclear compounds can be considered of homonuclear origin, where in another metal atom substitutes some metal atom. In the structural plan, within heteronuclear complexes, it is kept the triangle of the three metal atoms bound in the center to an atom of O^{2-} as a μ_3-oxo bridge.

The substitution of an iron atom within Fe_3O carcass with a cobalt atom leads to essential increase of quadrupole splitting value, and to the decrease of isomer shift value. Researchers [84] explain these changes within Mössbauer spectra through the fact that summary electron density around the iron atom nucleus increases, and the symmetry of the electron cloud around the iron nucleus decreases. Antiferromagnetic interactions among metal atoms can also be noticed within carcass compounds of Fe_2CoO.

An ample study of heteronuclear clusters of $[Fe_2M^{II}O(CH_3COO)_6L_3]\cdot nSolv$ (M=Co, Mn; L=derivatives of nicotinic and isonicotinic acids) is exposed in [85], that brings spectroscopy data in IR and Mössbauer, magnetochemistry and thermogravimetry.

There were achieved a series of studies on the influence of certain chemical compounds of different classes (carboxylates, dioximates, compositions of various compounds.) on the physiological parameters determining qualitatively and quantitatively crop productivity [86, 87].

The spectrum of homonuclear compounds can be extended by substituting water molecules from the internal coordination sphere with neutral organic ligands, including biologically active ligands, while the spectrum of heteronuclear compounds can be extended by varying the bivalent metal nature.

By substituting the different number of water molecules in $[Fe^{III}_3O(CH_3COO)_6(H_2O)_3]NO_3$ with derivatives of nicotinic and isonicotinic acids, there were obtained and studied clusters with the following general formula: $[Fe_3^{III}O(CH_3COO)_6(H_2O)_{3-n}L_n]NO_3 \cdot mSolv$, wherein L= N,N`-diethylnicotinamide (DENA), ethyl ester of nicotinic acid (EENA) or of isonicotinic acid (EEiNA); Solv = H_2O or CH_3COOH [77, 85]. On the basis of elements analysis, IR and Mössbauer spectroscopy and magnetic measurements data, it was concluded that the substitution of water molecules from internal coordination sphere with organic ligands does not affect the basic carcasses $\{Fe_3O\}$ or $\{Fe_2MO\}$ of clusters. It was also established that the use of homo- and heteronuclear carboxylates of μ_3-oxo type as stimulators of physiological processes in some crops have beneficial results and can be recommended for increasing plant productivity in agriculture [86].

Our data has demonstrated that treatment with trinuclear clusters of Fe^{3+} and Co^{2+} contributes to a considerable increase of germination energy, plant growth and productivity. Details about investigation results regarding the effect of coordination compounds on biological performance of plants are described in the doctoral theses [88-90], the novelty being confirmed by patents [91-104]. Throughout investigations, it was pursued not only the effect of coordination compounds administration on plant biological performance in different environmental conditions, there were additionally studied the induced mechanisms of these substances in plant response reactions to environmental conditions, and the ensurance of biological performances.

In literature, there are relatively few data related to PhAS action, especially to those of coordination compounds type, on the content and composition of free amino acids in plant tissues. As a result of biochemical tests performed on plants of *Zea mays* L., seminally treated with trinuclear clusters $[Fe^{3+}Co^{2+}]$ and $[Fe^{3+}Co^{2+}CO]$, and subjected to hydric stress (Hs) action, conditioned by drought – experiences by the doctor of biology Valeria Vrabie (2001), there haven't been found differences in the composition of free amino acids. As compared to control (70% TWC), there were established only quantitative differences [90]. Within the roots of treated plants alike those of control-plants, the major contribution is brought by free amino acids from pyruvate, oxaloacetate and oxoglutarate families. The major share belongs to alanine, aspartic acid, methionine and serine. The plants treated with Trifeden (Tab. III.1, Fig. III.2) show a a high content of hydroxyproline and valine, while those treated with Difecoden show a high content of leucine.

Table III.1. Quantitative and qualitative composition of free amino acids (mkg·g^{-1} s.u.) extracted from the organs of *Zea mays L.* plants exposed to hydric stress action (3 Hs days) and to treatment with trinuclear clusters of Fe^{3+} and Co^{2+}.

Amino acids	Roots				LeavesVII-VIII				Leaves XI-XII			
	Trifeden		Dfecoden		Trifeden		Dfecoden		Trifeden		Dfecoden	
	Contr.	**Hs**	**Contr.**	**Hs**	**Contr.**	**Hs**	**Contr.**	**Hs**	**Contr.**	**Hs**	**Contr.**	**Hs**
Leu	0.304	3.7	0.17	4.2	0.16	5.1	0.098	3.9	0.06	1.9	0.03	1.5
Ile	0.279	3.4	0.96	2.4	0.15	4.6	0.064	2.5	0.11	3.7	0.07	3.4
Val	0.520	6.4	0.14	3.5	0.26	7.9	0.17	6.8	0.16	5.5	0.08	4.3
Lys	0.033	0.4	0.06	1.4	0.07	2.4	0.06	2.4	0.02	0.6	0.02	0.8
Ala	0.527	6.5	0.21	5.4	0.78	24	0.62	24.8	0.57	19.7	0.39	20.3
Σ	**1.663**	**20.4**	**0.67**	**17.0**	**1.439**	**44.4**	**1.012**	**40.5**	**0.92**	**31.6**	**0.59**	**30,5**
Asp	1.282	15.7	0.36	9.4	0.062	1.9	0.054	2.2	0.07	2.45	0.05	2.6
Thr	0.317	3.89	0.15	3.9	0.175	5.4	0.132	5.3	0.09	3.2	0.06	3.4
Met	1.221	14.9	0.54	14	0.159	4.9	0.138	5.5	0.09	3.4	0.05	2.8
Σ	**2.82**	**34.6**	**1.06**	**27.0**	**0.396**	**9.1**	**0.324**	**12.9**	**0.26**	**9.1**	**0.17**	**8.8**
Gly	0.174	2.13	0.06	1.7	0.183	5.6	0.111	4.4	0.16	5.7	0.99	5.1
Ser	0.601	7.3	0.25	6.3	0.45	14	0.342	13.7	0.53	18.3	0.28	14.4
Cys	0.47	5.76	0.37	9.4	0.163	5.03	0.224	8.9	0.37	12.7	0.18	9.2
Σ	**1.245**	**15.2**	**0.68**	**17.0**	**0.8**	**24.7**	**0.67**	**27.1**	**1.06**	**36.7**	**0.73**	**37,4**
Glu	0.237	2.9	0.1	2.5	0.088	2.7	0.09	3.76	0.2	6.8	0.07	3.7
Arg	0.162	1.98	0.12	3.2	0.015	0.46	0.005	0.2	0.004	0.13	0.002	0.1
Pro	0.034	0.41	0.05	1.3	0.116	3.6	0.095	3.8	0.12	4	0.08	4.2
Hyp	1.77	21.7	0.93	2.4	0.109	3.4	0.125	5	0.21	7.2	0.19	9.8
Σ	**2.203**	**27**	**1.21**	**31.0**	**0.328**	**10.1**	**0.32**	**12.7**	**0.53**	**18.2**	**0.35**	**17.8**
Phe	0.07	0.85	0.08	2.1	0.145	4.5	0.095	3.8	0.07	2.5	0.05	2.6
Tyr	0.108	1.32	0.15	3.9	0.098	3	0.063	2.5	0.05	1.7	0.03	1.4
Trp	0.017	0.21	0.012	0.3	0.02	0.6	0.007	0.3	0.035	1.2	0.03	1.3
Σ	**0.195**	**2.4**	**0.25**	**6.4**	**0.263**	**8.1**	**0.165**	**6.6**	**0.16**	**5.5**	**0.11**	**5.4**
Hys	0.023	0.28	0.042	1.1	0.018	0.5	0.005	0.2	0.007	0.24	0.007	0.4
Total content	**8.149**	**99.89**	**3.92**	**99.5**	**3.24**	**96.9**	**2.502**	**100.0**	**2.94**	**101.3**	**1.95**	**100.3**

The content of amino acids of pyruvate and oxoglutarate groups exceeds control I (non treated plants 70% TWC) and control II (non treated plants on a drought background 30% TWC). It takes place the increase of free amino acids generated from products of Kalvin cycle, in particular of cysteine. These changes can be explained by the fact that under the action of Co^{2+} ions, it produces the activation of key-enzymes of aromatic amino acids synthesis. In line with this, it can be assumed that the high content of aromatic amino acids in plant roots can be conditioned namely by the activation of enzyme systems responsible for the synthesis of these amino acids. In the young leaves (XI-XII) of the same plants the major contribution belongs to amino acids of pyruvate family and Kalvin cycle.

As compared to control plants (70% and 30% TWC), after 3 days of humidity deficit, the treated plants showed an increase of the content of oxoglutarate family amino acids, basically on proline and hydroxyproline account. A large percentage belongs to alanine, valine, serine, cysteine and glycine. The content of aromatic amino acids does not significantly change.

Quantitative redistribution of amino acids within families according to origin in treated plants is the same as in the control. Both in the roots of treated plants, and in those of control plants, the major share belongs to alanine of pyruvate family. In roots and mature leaves, methionine contribution increases as compared with other amino acids derived from oxaloacetate.

In young leaves, it is kept the same quantitative ratio as compared to control plants (70% TWC). In leaves, as opposed to roots, in all studied variants, serine represents the major amino acid among amino acids derived from products of Kalvin cycle. Yet, in the roots of treated plants, it increases the contribution of cysteine. Within shikimate and oxoglutarate family, it is kept the same quantitative ratio as compared to control plants (70% TWC).

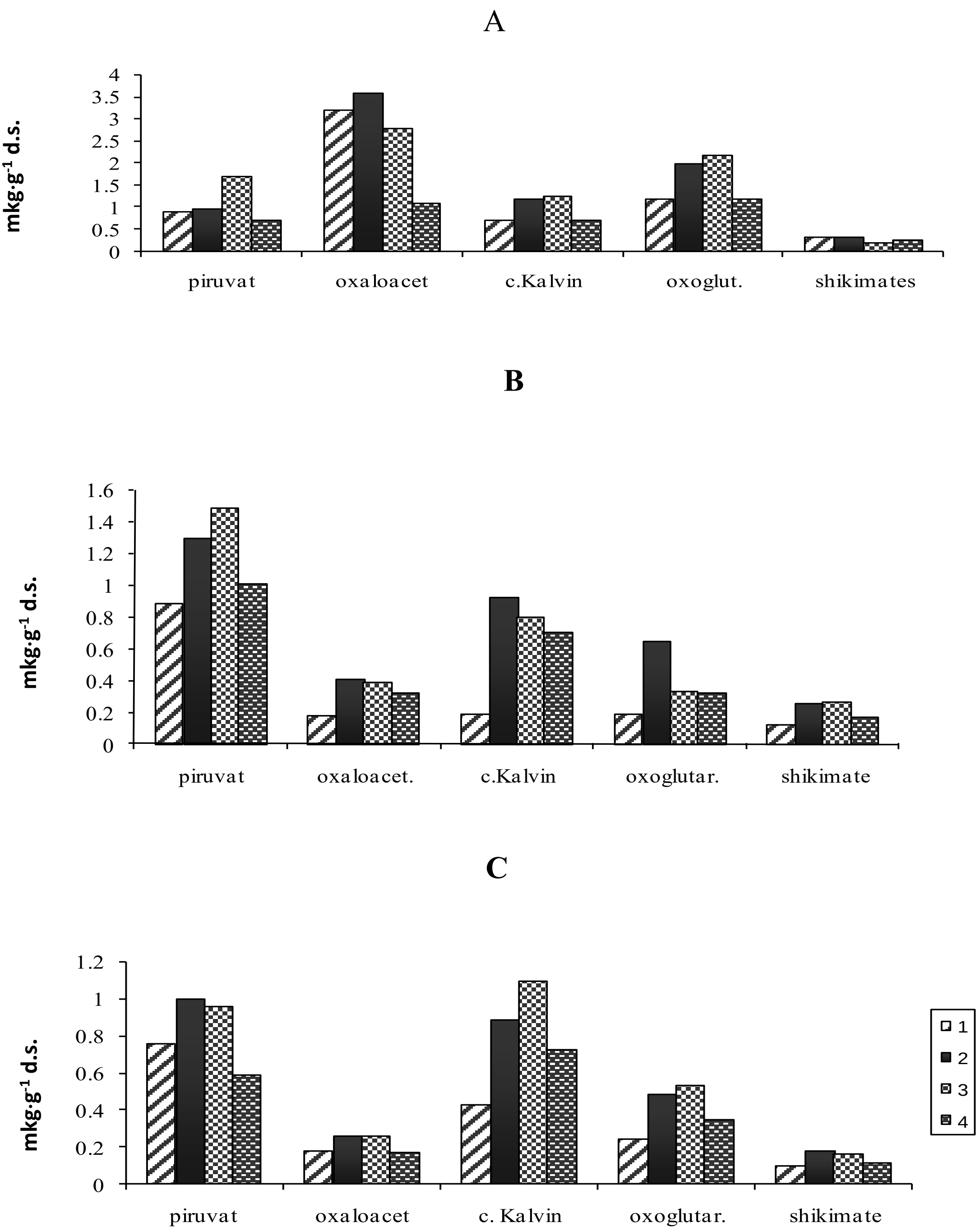

Fig. III.2. The action of Sh and trinuclear clusters [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}CO$] on the content of proteinogenous free amino acids according to their origin in roots (A), leaves VII-VIII (B); leaves XI-XII (C) of *Zea mays* L. plants. Legend: 1 – control (70% total water capacity of soil, TWC); 2 – drought (30% TWC) 3 – drought +Trifeden; 4 – drought + Difecoden.

Therefore, the obtained results lead to the conclusion that trinuclear clusters [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}CO$] condition the increase of amino acids content, deriving from Kalvin cycle products, in particular of cysteine and serine, in the organs of *Zea mays* L. plants. Trefeden, and particularly Difecoden contribute to the increase of amino acids originating from pyruvic acid, oxoglutarate and oxaloacetate, and from aromatic amino acids – phenylalanine and tyrosine. No significant differences were detected in the content of tryptophan, which probably stems from the fact that Co^{II} ions accelerate tryptophan transformation in auxine.

In the same study it was documented that the seed pre-treatment with trinuclear clusters of diethylnicotinamide with [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}CO$] increases the pattern content of readily soluble proteins (RSP) in the organs of corn planlets, under optimal conditions of humidity, and maintains it at a stable level during drought conditions. Qualitative and quantitative changes of RSP induced by trinuclear clusters with Fe^{3+} and Co^{2+}, at the initial stages of growth, maintain in the organs of mature plants, allowing the formation of a particular phenotype of plants resistant to drought.

The data obtained by V. Vrabie (2001) have shown that coordination compounds affect the electrophoretic spectrum and the relative activity of certain enzymes. It was established that in drought conditions, peroxidase activity (PX) in plant organs pre-treated with the studied compounds, as compared with the untreated ones, equals by value to that specific of control plants (70% TWC). During the drying period (1-7 days) PX activity in the treated plant roots increased 1.9 and 1.5 times, while in the untreated ones, increased 1.8 to 3.3 times (Fig. III.2).

Within leaves, it can be observed the same stabilizing action of [$Fe^{3+}Co^{2+}CO$] on PX activity. After 1-3 days of dehydration, PX isoenzymatic spectrum from leaves and roots seminally pretreated with [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}CO$] showed the same isoenzymatic composition as the PX spectrum of control plants organs (70% TWC) (Fig. III.3). There were, however, recorded changes in the relative activity of some isoenzymatic components of PX.

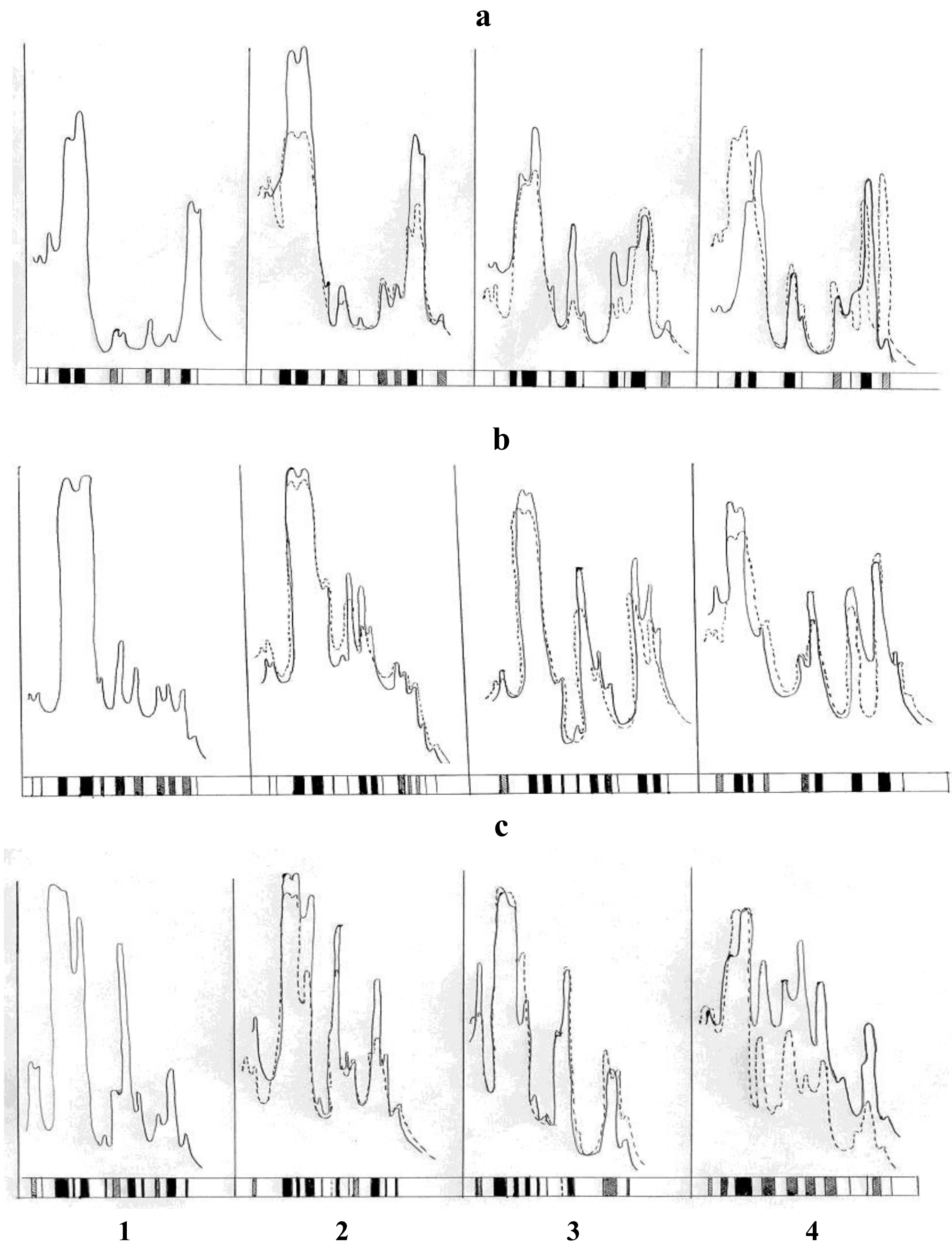

Fig. III.3. Electrophoregrams and density grams of peroxidase from roots (a) mature leaves (b) and young leaves (c) of *Zea mays* plants L. treated with [Fe^{3+}Co^{2+}] and [Fe^{3+}Co^{2+}CO] trinuclear clusters subjected to drought action.

Legend: 1 – control; 2-3 – Hs days; 3-7 – Hs days; 4-7 – reversion days;
------ Difecoden action during drought.

After 3 Hs days, the relative activity of isoenzymatic components in the roots of plants treated with trinuclear clusters increased by Rf: 0.36 and 0.39. On the seventh day of drought, it can be observed *de novo* the occurance of isoenzymatic components with Rf 0.26 and 0.49. Within the isoenzymatic spectrum of PX from the roots of untreated plants (30% TWC), these components appear after 3 days of water deficit. In mature leaves, in the early days of dehydration, the relative activity of isoperoxidase components increases by Rf 0.05; 0.08; 0.125 and 0.18. Within the isoenzymatic spectrum of PX from mature leaves of plants treated with Trifeden, as compared to those treated with Difecoden, alike untreated ones, after 7 days of drought, it can be identified the emergence of a new component with Rf 0.18.

The plants of this variant show an increase of isoenzyme relative activity by Rf 0.28; 0.29; 0.32; 0.33, 0.35. The effect of trinuclear clusters [$Fe^{3+}Co^{2+}$] şi [$Fe^{3+}Co^{2+}CO$] in young leaves, on a Hs background, can be characterized by the increase of isoenzymes relative activity with Rf 0.08; 0.125; 0.19; 0.28; 0.295 and 0.33. Both in the treated and in the untreated plants, PX isoenzymes with Rf 0.175 and 0.295 PX have a maximum peroxidase activity. Yet, within the organs of plants treated with the studied compounds, unlike the untreated ones, PX activity equals by value to that specific of control plants (70% TWC).

During dehydration period (1-7 days), within the roots of treated plants, PX activity has increased by 1.9 and 1.5 times, while within untreated ones, it has increased by 1.8 to 3.3 times. Within leaves, it can be observed, the same stabilizing action of [$Fe^{3+}Co^{2+}CO$] on PX activity. Within young leaves, however, under the influence of Trifeden, it was detected a more significant increase of PX activity as compared to untreated plants. After 1-3 days of dehydration the isoenzyme spectrum of PX from the leaves and roots of plants treated seminally with [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}CO$] was characterized by the same composition as the PX spectrum from control plants organs (70% TWC). There were, however, changes in the relative activity of some isoenzymatic components of PX.

After 3 Hs days within the roots of plants treated with trinuclear clusters, the relative activity of isoenzymatic components increased by Rf 0.36 and 0.39. On the seventh drought day it is observed *de novo* the occurance of isoenzymatic components Rf 0.26 and 0.49. Within the isoenzymatic spectrum of PX from the roots of untreated plants (30% TWC) these components appear after 3 days of water deficit. In mature leaves, in the early days of dehydration, it occurs the increase of isoperoxidase components relative activity with Rf 0.05; 0.08; 0.125 and 0.18. Within the isoenzymatic spectrum of PX from mature leaves of plants treated with Trifeden, unlike those treated with Difecoden, as in the untreated ones, after 7 days of drought, it is identified the emergence of a new component with Rf 0.18. The plants of this variant show an increase of isoenzymes relative activity with Rf 0.28; 0.29; 0.32; 0.33, 0.35. Trinuclear clusters [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}CO$] under drought caused the increase of isoenzymes relative activity with Rf 0.08; 0.125 0.19; 0.28; 0.295 and 0.33 in young leaves. Both in treated and untreated plants PO isoenzymes with Rf 0.175 and 0.295 show a maximum peroxidase activity.

In the plants treated with the studied clusters during drought, It has been found a relatively high activity of cytochrome oxidase (CO) isoenzymatic components [90, 94]. Within the roots of treated plants, it is detected the same isoenzymatic composition of CO as in the control plants (70% TWC). It takes place the increase of CO components relative activity with Rf: 0.16; 0.22; 0.24; 0.3. Within the CO isoenzymatic spectrum of plant leaves treated with Difecoden unlike untreated ones after 3 days of Hs, it is recorded the intensification of isoforms activity with Rf: 0.22; 0.1; 0.12; 0.156. The high activity of CO components from roots and leaves maintains after 7 days of hydric deficit.

On rehydration, within the CO isoenzymatic spectrum of treated plant leaves, it is *de novo* recorded the occurance of the component with Rf 0.18. Therefore, within the organs of corn plants treated with trinuclear clusters of [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}CO$], during moderate drought (3 Hs days), it can be observed the stabilization of PX and CO isoenzimatic spectrum, but it diminishes as the drought performs in time. The emergence of new enzymatic components in the enzymatic spectrum from the organs of treated plants, unlike the untreated ones, is recorded after 7 days of water deficit, probably it occurs the modification of isoforms relative activity that fulfil in Hs conditions the biggest functional task.

Thus, the functional role of trinuclear clusters of [$Fe^{3+}Co^{2+}$] with Dena and [$Fe^{3+}Co^{2+}$CO] is achieved by keeping the RSP pattern quantitatively and qualitatively identical to that of unstressed plants. Trifeden and Difecoden, probably, contribute to RSP proteolysis diminuation, to polyribosomes cleavage and to a less impairment of metabolic reactions caused by dehydration. Stabilizing biochemical reactions and physiological processes in the plant body by maintaining the activity and electrophoretic spectrum of certain enzymes (PX, CO), the content of the most important amino acids, [$Fe^{3+}Co^{2+}$] and [$Fe^{3+}Co^{2+}$CO] trinuclear clusters favor the growth of plant potential resistance to drought stress.

There have been carried out researches regarding the argumentation of the posibility to exogenously adjust corn plants functional status, growth and productivity under conditions of mild drought. Through special experiences, it has been demonstrated that if drought coincides with the initial stage of organogenesis, crop losses are maximum because of disrupting the process of cells division, reducing germination energy, density of plants per unit area and accumulation of plantlets phyto-weight. This stage is one of the critical stages for plants. The results of many laboratory experiments have shown the beneficial and authentic effect of the studied trinuclear clusters on the speed of seed swelling, germination energy, mobilization and assimilates use from kernels, planlets growth and biomass accumulation, and the effect on rizogenesis and assimilating apparatus formation at initial stages of ontogenesis. It has been found that both the IAA and the Difecoden accelerate the rate and extent of seeds imbibition with water. Thus, hydration of corn seed biocolloids at imbibition phase constituted in „control” variant 46.24%; at seeds treatment with indolyl acetic acid – 48.9%, and at seeds treatment with Trifeden – 48.7%. The effect on biomass accumulation of plantlets was 15.48 % in the plantlets from seeds treated with choline chloride (analog I); 120.0% – in those from seeds treated with PhAS ethylene-diamine-disuccinic acid (proximate analog) and 132.10% as compared to control – seeds treated with Trifeden (Tab. III.2).

Table III.2. The effect of PhAS on corn growth at the initial stages of ontogenesis.

Variants, preparation, %	Length, mm			Growth index, $L_{col}/L_{col} + L_r$	Biomass, mg	
	coleoptile	rootlet	total of radicular system		coleoptile	root
	M ± m	M ± m	M ± m		M ± m	M ± m
control, H_2O	18.7 ± 0.9	54.4 ± 2.3	83.6 ± 3.6	0.256	145.6 ± 5.0	172.3 ± 4.8
IAA, analog	19.8 ± 1.0	56.3 ± 2.1	96.5 ± 3.0	0.260	182.2 ± 2.6	193.4 ± 3.6
CoEDDS, 0.001%	22.4 ± 0.9	61.0 ± 1.5	114.4 ± 2.6	0.268	200.5 ± 4.8	210.9 ± 6.2
Difecoden, 0.001%	29.5 ± 1.4	65.8 ± 1.6	116.6 ± 1.1	0.309	207.7 ± 5.9	215.7 ± 2.9

The major effect on the activation of vital processes in seed germination and growth of *Zea mays* L. plantlets was recorded while using Difecoden – 145.25 %. Seed germination energy, at a water potential in the substrate of only 1.5-1.7 Mpa in the control variant decreased 2.42 times, while in the seeds treated with Difecoden and Trifeden Eg, it was 1.4 to 1.5 times higher as compared to the control sample under the same conditions (Tab. III.2; Fig. III.4).

Osmoconditioning the seeds for sowing with trinuclear clusters, containing Fe^{3+} (Trifeden), especially Fe^{3+} and Co^{2+}, (Difecoden) ensured a higher growth index (G) of plantlets in conditions of „drought" (ψ_w -1 5 MPa).

$G(L_{col}/L_{col} + L_r)$ value in the plants of control sample constituted 0.387 under optimal conditions, and during „drought" – 0.37; in the plantlets from seeds treated with Trifeden – 0.438 and 0.37 correspondingly, and in those treated with „Difecoden" – 0.67 and 0.35. This is explained by the fact that in the first sample, along with the authentic increase of growth processes of the root system, coleoptile growth is equally higher, as compared to coleoptile growth in control plants (Fig. III.4).

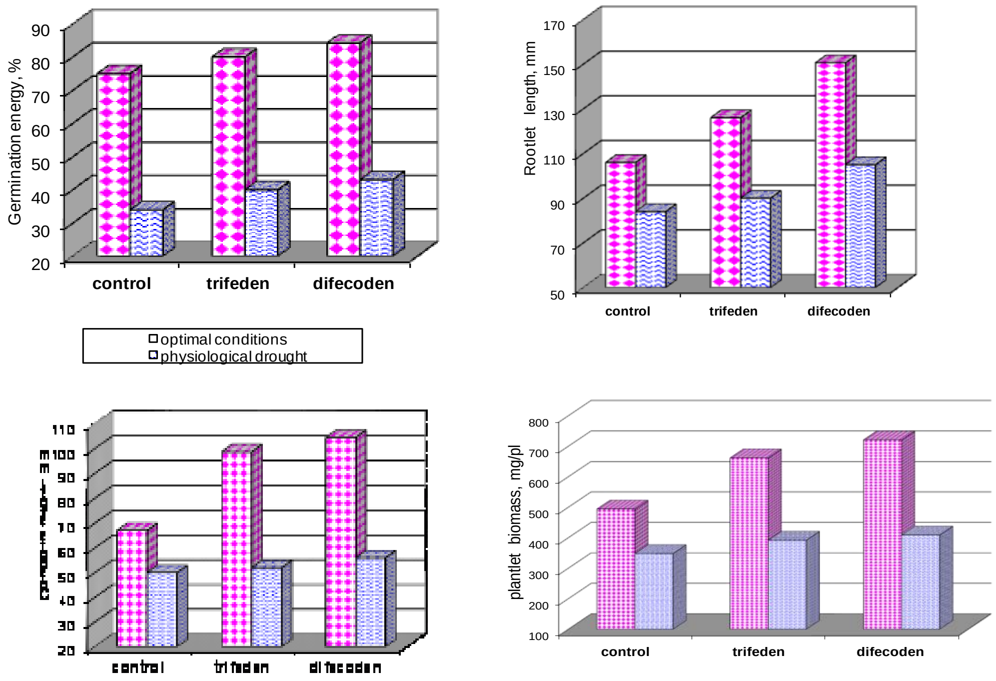

Fig. III.4. The effect of seeds treatment with physiologically active substances on *Zea mays* L. germination and growth at the initial stages of ontogenesis in optimal conditions and reduced humidity.

Along with coleoptile linear growth intensification, its biomass exceeded the biomass of „control" variant plantlets. Biomass accumulation in the latter exceeded by 20% the process of plantlet biomass accumulation from control variant.

It should be noted that Difecoden has a beneficial influence on the activity of start reactions in the germination of *Glycine max* Merr (L.) seeds. If using indolyl acetic acid (proximate analog), the rootlet growth rate was 1.24-1.3 times higher as compared to the control variant, then in the plantlets from seeds treated with Difecoden, this index exceeded 1.3-

1.4 times the growth rate of plantlets from „proximate analog" variant. An increased rate of biomass accumulation in these plantlets was also recorded in the true third leaf stage. It was 54.3 mg m.u. day^{-1} in control plants, 61.4 – in the plants treated with IAA and de 61.6-62 mg m.u.·day^{-1} – in the plants treated with aqueous Difecoden [91].

The use of Fe^{3+} coordination compounds, especially of those containing Fe^{3+} and Co^{2+}, contributed to a veracious increase of pigments fund in leaves (Fig. III.5).

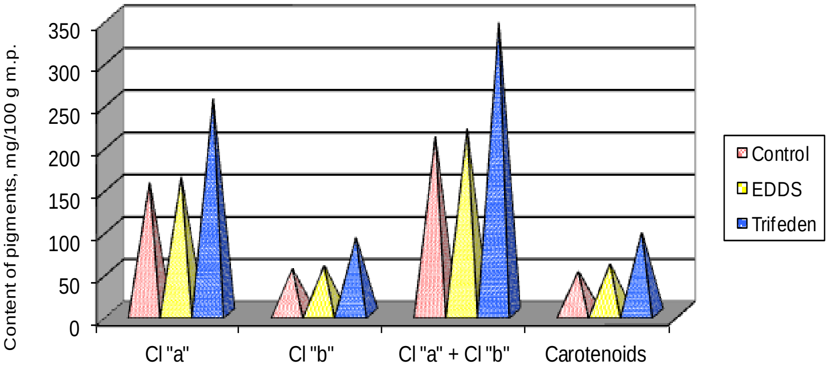

Fig. III.5. The content of assimilating pigments in corn leaves.

Thus, in the plant leaves of *Zea mays* L. cv. M 456, the summary content of green pigments marked an increase of 12-15%; in cv. 425 M, the background of „chlorophyll *a* + chlorophyll *b*" was 344.95 mg·100 g^{-1} m.p. in the variant „treated with Trifeden" and 208.77 mg·100 g^{-1} m.p. – in the control variant. Parallelly, it was recorded an increase of carotenoids content by 30.7 and 70.6%.

The content of assimilating pigments in the leaves of plants treated with trinuclear clusters containing Fe^{3+} and Fe^{3+} and Co^{2+} heteronuclears ensured a better functioning of the photosynthetic apparatus, which resulted in a higher primary productivity. In the plants of *Glycine max* Merr L., cv. Bucuria, it was recorded the same beneficial effect of Difecoden (Tab. III.3) on biological performances.

Table III.3. The influence of soybean seeds treatment with PhAS on growth and photosynthesis processes of *Glycine max*, Merr (L.), cv. Bucuria plants

Variants	Plant biomass, g (m.u.)	Rate of biomass accumulation, mg/zi	Modification degree under stress conditions	Leaf area dm^2/ plant	Modification degree under stress conditions	EAA, $mg/dm^2/day$	Modification degree under stress conditions
Temperature action in the third leaf stage							
Control	2.17 ± 0.05 2.56 ± 0.4	54.3 40.6	-3.17	1.37 ± 0.08 1.70 ± 0.02	+0.3	78.8 26.4	-52.4
IAA, 0.0001%	2.46 ± 0.03 3.10 ± 0.04	61.4 64.2	+2.8	1.46 ± 0.05 2.48 ± 0.04	+1.0	84.0 32.6	-51.4
Difecoden, 0.001%	2.45 ± 0.06 3.22 ± 0.03	61.6 75.5	+13.8	1.65 ± 0.02 2.64 ± 0.02	-1.0	74.6 35.1	-39.4
Temperature action in the anthesis stage							
Control	5.55 ± 0.08 6.08 ± 0.21	89.5 66.7	-22.7	3.27 ± 0.06 3.67 ± 0.18	+0.4	55.5 19.2	-36.2
IAA, 0.0001%	5.46 ± 0.09 6.27 ± 0.13	88.1 101.7	+13.7	3.52 ± 0.05 4.23 ± 0.13	+0.7	57.0 27.8	-29.2
Difecoden, 0.001%	5.96 ± 0.07 7.03 ± 0.25	96.4 131.2	+34.8	3.57 ± 0.04 4.49 ± 0.17	+0.9	54.1 32.6	-21.4

Biomass accumulation, formation and efficiency of assimilating apparatus (EAA) in plants treated with Difecoden were higher than in the control variant. The obtained information about the effect of PhAS of complex type on physiological processes at initial stages (laboratory experiments) served as an argument supporting the hypothesis about achieving the stimulating effect on the elements associated with the productivity of plants grown under natural conditions of humidity. The main indicator characterizing the plant economic value is productivity. The specificity of achieving the productivity potential is determined by climatic conditions during the growing season, and by plants trophic insurance.

Large yields are ensured through the optimal combination of all elements of productivity [105]. It has been found that there is a strong positive correlation between plants productivity, grain weight and number per ear, leaf area, duration of photosynthetic apparatus activity and plant weight.

Plant productivity is most closely correlated with plant leaf area or with its photosynthetic potential [106, 107]. Parameters of leaf area and leaf distribution character are assigned to the main factors limiting the quantity of plant production. [108]. It was revealed that yield always correlates with the total leaf area [109]. At the same time, it is known that the stem serves as a deposit of assimilates for grain growth, it is a buffer zone that maintains water status in the cob at a favorable level [110]. Therefore, in areas with periodic and moderate droughts, it is advantageous to maintain leaf growth and accumulation of plant phytoweight at an optimal level in order to prevent yield reduction.

The results of our investigations have revealed authentic differences regarding the action effect of trinuclear clusters with Fe^{3+}, Fe^{3+} and Co^{2+} (Trifeden and Difecoden) and of coordination compounds, traditionally used in agriculture – ethylenediamine disuccinates (CoEDDS), on the formation of productivity elements in *Zea mays* L. (Tab. III.4).

Table III.4. The influence of physiologically active substances on the formation of productivity elements of corn plants.

Variants	Height, Cm		Leaf area, m^2/pl		Biomass, g/pl		Grain yield, q/ha	
	M±m	*td**	M±m	*td*	M±m	*td*	M±m	*td*
Control	163.7 ± 3.2		0.524 ± 0.01		283.0 ± 8.4		79.4 ± 1.2* 55.0 ± 1.5**	
CoEDDS	154.7 ± 2.4	td_1 2.2	0.629 ± 0.03	3.3	349.4 ± 9.8	5.1	84.2 ± 1.3 57.4 ± 1.1	2.7 1.3
Trifeden	189.1 ± 1.8	td_2 6.9 td_3 11.6	0.656 ± 0.01	12.3 2.2	421.3±6.9	12.7 6.0	99.4 ± 1.6 80.0 ± 1.5	10.1 11.8 7.5 12.2

Note: td_1 – authenticity of difference between CoEDDS (the closest analog) and control; td_2 – authenticity of difference between Trifeden and control, td_3 – authenticity of difference between Trifeden and CoEDDS (proximate analog);

* optimal conditions of humidity

** drought conditions

The obtained results reflect a cause-effect interconnection between the treatment of seeds before sowing and plant biological performance: plant habitus, leaf area, phytoweight accumulation and grain yield.

Seed treatment with Trifeden conditioned a considerable increase in growth processes and habitus by 15% as compared to control plants (untreated plants) and by 22% in comparison with plants treated with CoEDDS. The mean leaf area of plants treated with CoEDDS constituted 120% as compared to control; and that of plants treated with Trifeden – 125%.

The research data on the formation of corn plant productivity reflect its dependence on the nature of preparation used to treat seeds before sowing.

A major effect was recorded while using Difecoden (Tab. III.5). The Difecoden possesses features of PhAS and contributes to the intensification of growth processes, development and formation of plant productivity elements. Seminal treatment with Difecoden has a significant positive influence on plant growth in height, foliage formation, phytoweight accumulation, and on grain yield.

Table III.5. The influence of physiologically active substances on the formation of elements of corn plant productivity.

Variants	Average height of a plant at the moment of crop, cm	Total phytoweight, g	Plant average vegetative weight, g	Cob average weight, g	Grain yield at 14% humidity q/ha
Control	163.67 ± 3.20	288.9 ± 15.17	150.9 ± 0.97	138.9 ± 14.2	55.02 ± 4.61
CoEDDS, proximate analog	154.76 ± 2.30	353.4 ± 9.73	211.5 ± 1.49	141.9 ± 8.24	59.80 ± 2.93
Difecoden	12.82 ± 2.55	578.9 ± 11.98	290.9 ± 1.31	287.9 ± 10.67	106.99 ± 4.10

The plants seminally treated with Difecoden showed a higher quantity of phytoweight accumulation and cobs, unlike the variant treated with Trifeden. Grain yield from the plants of Difecoden variant, recalculated to ha at 14% standard humidity, constituted 194.45%, and in the „proximate analog” – 108.75% as compared to control. The effect of treatment with Difecoden is significantly veracious after the first level of probability (Tab. III.6). At the same time, it was established the possibility of improving corn plant resistance to a soil humidity deficit caused by natural drought. The data obtained confirms the possibility to improve plant resistance to a relative water insufficiency in the soil. The plants from seeds treated with Difecoden distinguish by a more intensive growth, that ensures a better formation of leaf area and biomass accumulation.

In the plants untreated with PhAS, drought reduced the assimilation area by 10.7% and biomass by 30.85%; in Trifeden treated plants, drought action constituted: 6.3 and 12.4%; while in the plants treated with Difecoden, these indexes differed slightly from control plants on irrigation background. The major cause for ceasing the accumulation of biomass in plants exposed to water stress, caused by water deficit in the soil, is not only a consequence of decreasing water absorption. It is also a consequence of reducing the accessibility of nutrients from the soil, since it reduces, as noted above, transpiration rate.

Table III.6. The influence of treating *Zea mays* L. seeds with PhAS on the formation of plant productivity elements under conditions of reduced humidity.

Variants	Growth conditions	Plant height, cm	Leaf area, m^2/plant	Plant biomass, g	Grain yield, q/ha
Treatment with H_2O	optimal (control)	163.7 ± 3.2	0.524 ± 0.01	283.0 ± 8.4	55.0 ± 5.1
	drought	126.2 ± 1.8	0.468 ± 0.03	172.9 ± 6.1	21.8 ± 2.8
Treatment with Trifeden	optimal	189.1 ± 1.8	0.656 ± 0.01	421.3 ± 6.9	80.8 ± 4.3
	drought	130.9 ± 1.0	0.480 ± 0.02	264.9 ± 4.1	29.3 ± 2.0
Treatment with Difecoden	optimal	192.8 ± 2.5	0.741 ± 0.08	578.9 ± 11.9	106.9 ± 4.1
	drought	135.3 ± 1.3	0.502 ± 0.09	275.4 ± 8.6	34.3 ± 6.7

In addition to this, growth inhibition in conditions of limited humidity is also caused by the decrease of assimilated carbon rate. However, reducing the number and size of leaves has the highest importance on achieving both primary and valuable agricultural part productivity. Plants phenotype that formed through the administration of bioactive substances on the basis of galates, salicylates, and nicotinamide differed by a more complete deployment of leaf area potential. As a result, the plants from seeds treated with Difecoden ensured a greater yield. Yield reduction under humidity deficit constituted about 60.4% in plants untreated with PhAS, 46.7% – in plants treated with Trifeden and 41.0% – in plants treated with Difecoden. In field experiments, under conditions of natural humidity, it was established a major effect on biomass accumulation in plants treated with Difecoden.

Water-soluble polymers enhance the effect of bioactive substances, thus increasing plant habitus. It was demonstrated the positive influence of polyvinylpyrrolidone on the formation of leaf area, biomass accumulation and plant yield (Tab. III.7; Fig. III.6).

Table III.7. The influence of PhAS on biomass and leaf area of *Zea mays* L. plants. Field experience, „paniculation-flowering" stage.

Cultivation	Variants	Biomass, g/pl		Leaf area, dm^2/pl		Yield, q·$ha^{-1,}$ at 14% humidity	
		M ± m	Δ%	M ± m	Δ%	M ± m	Δ%
M 291	Control	508.37 ± 24.3		85.66± 2.5		68.90 ± 1.9	1.62
	Control + PVP	699.86± 30.0	37.67	98.96 ± 4.5	15.53	75.70 ± 2.9	1.90
	Difecoden	699.39± 6.5	37.57	96.54± 3.6	12.70	80.18 ± 3.7	1.77
	Difecoden +PVP	719.13 ± 22.7	41.46	90.64 ± 0.1	5.60	80.07 ± 1.2	1.97
P 459	Control	417.35± 15.5		90.94± 2.9		83.89 ± 0.6	2.72
	Control + PVP	494.78 ± 19.9	18.55	94.78 ± 0.4	4.22	86.67 ± 0.8	3.28
	Difecoden	578.11± 18.2	38.52	98.32± 4.5	8.12	93.63 ± 2.4	2.84
	Difecoden + PVP	592.42± 17.6	41.95	87.14 ± 2.3	-6.91	96.52 ± 4.7	3.47

The use of polyvinylpyrrolidone (PVP) as a an antitranspirant in combination with PhAS, in particular with Difecoden, contributes to a veracious increase of plant growth in height and vegetal weight accumulation. The yield index (grain weight relative to plant weight) was higher in plants treated with PhAS.

Therefore, exogenously administered bioactive compounds, along with the growth of plant vegetal weight, condition an increase in the share of assimilates used to form grain yield.

Control Difecoden Difecoden + PVP

Fig. III.6. The influence of PhAS on the formation of leaf area of *Zea mays* L., cv. P 459 plants. Field experience.

Water-soluble polymers used as antiperspirants increased the effect of bioactive substances. Above-ground total weight of the plants treated with the combination of PhAS + polymer exceeded the value of this index.

Thus, trinuclear coordination compounds containing Fe^{3+}, Fe^{3+} and Co^{2+} possess characteristics of bioactive substances, with growth regulators features. The treatment of sowing seeds and foliage apparatus during vegetation conditions the optimization of plant functional state, growth and development, both under favorable conditions of humidity and in conditions of moderate water deficiency. PhAS under use can activate vital processes at the very initial stages of plants individual development, stimulate the root system and shoot growth, ensure water status homeostating under a suboptimal hydric regime, increase plants productivity, strengthen body protective functions by increasing tolerance to suboptimal factors. The use of PhAS, containing in their molecule nutrients, ensures the stabilization of plant production process. The joint use of water-soluble polymers (PVP, coVP) with PhAS helps to reduce water consumption in the process of perspiration without negative consequences on plant biomass accumulation.

In order to increase corn plant productivity and yield, it was developed a proceeding of using Coditiaz coordination compound. The coordination compound with the chemical formula $[Co(DH)_2(Thio)_2]NO_3$, conventionally – Coditiaz, is a coordination compound of cobalt(III), on the basis of dimethylglyoxime and thiocarbamide, in the form of a brown crystalline substance, soluble in water, methanol and ethanol, and insoluble in diethyl ether. The beneficial effect of this compound was documented in several series of laboratory and vegetation experiments. It was studied the effect of seeds treatment before sowing and that of plants treatment during vegetative growth, at the V-VII-th leaf stages, on the growth and development of *Zea mays* L., cv P 459 and X5P515 plants.The scheme of experiences included the following variants: 1 – control, plants from seeds treated with water; 2 – plants treated with aqueous Coditiaz. In order to detect the range of Coditiaz optimal physiologic concentrations, it was investigated the influence of solutions at 0.0001 to 0.1% concentrations on plant vital processes at the initial stages of ontogenesis. The results are shown in Table III.8.

Experimental data show the capacity of used substances to stimulate plant growth already at the initial stages of ontogenesis. The use of Coditiaz as a growth stimulator conditions a better plant development. The effect of seed treatment according to invention constitutes an increase of plant weight by 19.6-32.1% at the initial stages of ontogenesis. A major influence is ensured by seed treatment with Coditiaz solution at a concentration of 0.0001; 0.0005 and 0.001%.

Within these variants, it was recorded the intensification of growth processes by 23.2 and 32.1% as compared to control plants.

Table III.8. Morphological parameters of corn plantlets from seeds treated with PhAS.

Variants	Concentr., %	Weight, mg			
		Coleoptile	Roots	Plantlet	Efficiency, % control
Control		220±5.0	340±14.0	560±7.0	100
Coditiaz	0.1	80±2.0	80±3.0	160±6.0	28.57
	0.01	190±6.4	270±5.4	460±20.0	82.14
	0.001	280±10.4	390±13.8	670±17.5	119.64
	0.0005	310±14.5	430±16.0	740±27.0	132.14
	0.0001	290±8.2	400±15.5	690±16.0	123.21

In vegetation experiences, it was studied the influence of Coditiaz on the content of assimilating pigments, plant growth and development. It was established (Tab. III.9) that the treatment of sowing seeds and plants during vegetative growth with Coditiaz solution of 0.0005% ensures the photosynthetic apparatus formation with an increased content of assimilating pigments: *a* chlorophyll content increased by 23.71%, and carotenoids content increased by 56.3%.

Table III.9. The content of assimilating pigments (mg/100 g subst.) in corn plants leaves treated with Coditiaz.

Variants	Chlorophyll *a*		Chlorophyll *b*		Carotinoids	
	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control
Control	164.79±0.09		55.25±0.15		47.98±0.26	
Coditiaz, 0.0005%	203.86±0.60	23.71	77.85±0.31	40.90	74.99±0.46	56.29

The formation of assimilating apparatus with a high content of pigments had positive effects on plant growth and development (Tab. III.10).

Table III.10. Morphological parameters of corn plants treated with Coditiaz.

Variants	Plant height, cm		Leaf area, dm^2		Aerial part weight, g		Radicular system weight, g		Efficiency
	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %	%
Control	150.8±1.2		25.9±0.2		229.9±2.1		75.0±0.8		100
Coditiaz	181.8±1.1	20.5	30.4±0.8	17.0	263.3±2.6	14.6	90.1±1.1	20.1	117.4

Plant treatment with Coditiaz coordination compound ensured the formation of more vigurous plants, with a better developed leaf area and radicular system, fact that had a veracious influence on biomass accumulation and fuller achievement of productivity potential (Tab. III.11).

Table III.11. Productivity and structure of the yield of corn plants treated with Coditiaz.

Variants	Cob weight, g/plant		Productivity, g/plant		Number of grains, pieces/plant		Weight of 1000 grains, g	
	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %
Control	98.3±0.4		70.7±0.6		265.8±1.7		266.1±1.2	
Coditiaz	117.0±0.8	19.0	88.0±0.7	24.4	309.5±2.1	16.4	284.3±1.9	6.8

Investigation results demonstrate that treatment with Coditiaz solutions condition a veridical plant productivity increase by 24.4% as compared to control plants. Therefore, seed treatment prior to sowing and plants treatment during flowering with Coditiaz aqueous solution in a concentration of $0.0005\%_{mas}$ stimulates the growth process, ensures the formation of a more efficient assimilating apparatus, the accumulation of plant weight and productivity increase.

The influence of PhAS on Cucumis sativus, L. and Lycopersicon esculentum L. Plant growth and productivity

For vegetable growing regions, during plant active growing, adverse conditions, most often of climatic origin (humidity deficiency, intense heat, cold, salinity, etc.) are frequent phenomena. Although these crops are usually planted on the best soils, it is almost impossible to exclude the influence of bad weather.

Adverse environment conditions reduce yield and worsen its quality. The prospects of mitigating or liquidating extreme factors impact on plant productivity remains one of the current tasks of vegetable gardening. A sparsely investigated field is the influence of unfavorable factors on the change of yield structure components and the possibilities of exogenously regulating the charge of plant fructification under ecological risk conditions (climatic). However, the few data published in recent years demonstrate that the structural elements of yield (number of fertile shoots, number of tubers, number of fruit on the shoot, fruit weight, etc.), first and differently react to adverse conditions and may have a different revocability to exogenously regulatory factors, such as the PhAS.

Given the fact that vitamins, including those of group B, are factors of growth processes trophic regulation due to their involvement in metabolic reactions in roots and shoots, we considered that the supplemental administration of PP vitamin in the form of nitrate of tris-nicotinamide-hexaacetate(O,O′)-μ_3-oxo-triiron(III)trihydrate (conventionally Trifenamid) will foster the processes of growth, development and productivity of *Cucumis sativus* L. and *Lycopersion esculentum* L. plants – crops requiring the least noxious cultivation technology from the ecological point of view. Investigations based on the assumption that the studied substances, containing in their molecule vitamin PP could exert an activation effect on growth processes. As is well known [14], the biochemical role of vitamin PP consists in its participation as a coenzyme (NAD and NADP) in the large group of oxidoreductases (dehydrogenases), which catalyzes over 150 biochemical reactions of dehydrogenation, oxidation N-alkylation, isomerization, reduction of nitrate into nitrite and further to ammonia in photosynthesis, respiration, energize reactions, anaerobic cleavage of carbohydrates etc.

Testing the effect of different physiologically active substances on the formation of plant productivity elements and on the agriculturally valuable part of production, revealed the veraciously positive effect of the nitrate of *tris*-nicotinamide-hexaacetate(O,O′)-μ_3-oxo-triiron(III)trihydrate, conventionally called Trifenamid [99]. Data obtained in laboratory experiments (Tab. III.12) demonstrated that both the Trifeden and Trifenamid accelerate the growth processes of *Cucumis sativus* L. plants at the initial stages of ontogenesis.

The beneficial effect of Trifenamid consists in increasing germination energy of seeds, accelerating rhizogenesis, plant biomass accumulation, and the ramification degree of adventitious roots.

Table III.12. The influence of PhAS on the growth of *Cucumis sativus* L. plants at the initial stages of ontogenesis
(average of 5 laboratory experiences).

Variants		Germination energy, %	Plantlet biomass, mg m.p./pl.	Number of adventive roots	Length, cm		
					rootlet	hypocotyl	effect, % control
Control, H_2O		71.4 ± 3.0	181.15 ± 9.1	$\frac{3.5 \pm 0.2}{13.4 \pm 0.6}$	9.8 ± 0.7	5.1 ± 0.5	100
Trifeden	0.0001%	79.1±5.2	215.9 ± 4.5	$\frac{3.3 \pm 0.1}{13.7 \pm 0.5}$	9.6 ± 0.7	5.9 ± 0.5	115.2
	0.001%	80.9 ± 2.4	222.7 ± 8.8	$\frac{3.5 \pm 0.1}{14.3 \pm 0.8}$	10.4 ± 0.4	5.7±0.3	118.7
	0.01%	70.5 ± 5.5	190.7 ± 4.5	$\frac{3.2 \pm 0.1}{11.9 \pm 0.9}$	13.01 ± 0.7	7.4 ± 0.4	104.3
	0.1%	4.8 ± 4.9	181.49 ± 7.5	$\frac{3.0 \pm 1.1}{0}$	0.2 ± 0.03	1.4 ± 0.6	67.1

Trifenamid	0.0001%	73.4 ± 2.9	221.4 ± 7.2	3.0 ± 0.1 16.3 ± 0.8	8.6 ± 0.9	5.8 ± 0.3	115.5
	0.001%	85.7 ± 5.8	233.4 ± 5.2	3.5 ± 0.1 17.8 ± 0.5	11.4 ± 0.4	7.3 ± 0.4	126.3
	0.01%	71.5 ± 5.0	227.0 ± 6.6	3.73 ± 0.2 20.5 ± 1.6	11.0 ± 0.5	5.4 ± 0.7	119.3
	0.1%	14.3 ± 5.8	188.4 ± 10.7	2.1 ± 0.9 0	0.9 ± 0.4	3.4 ± 1.1	73.5

The treatment of *Cucumis sativus* L. cucumber seeds with Trifeden conditions the stimulation of plant growth at early stages by 14.8-18.7% as compared to control, while the treatment with Trifenamid provides a stimulus for growth by 15.5 to 26.3% (Fig. III.7).

The data obtained from field experiences and presented in Tab. III.13 and Fig. III.7, show that the treatment of seeds and foliar apparatus generate quantitative changes of some parts of cucumber and tomato plant productivity, thus affecting yield per unit area. Plant treatment with Trifeden stimulates fructification by 30-35%; fruit growth – by 7.5-8.0%, fact ensuring an increase in plant productivity by 38.7%, as compared to control plants. A major effect was obtained while using Trifenamid.

Table III.13. The influence of PhAS on the productivity of cucumber and tomato plants.

Variants	Number of cucumbers from 10 plants, unit.	Average weight of 1 cucumber, g	Productivity, $g \cdot pl.^{-1}$	Yield, $g \cdot 1\ m^2$
Cucumis sativus, L., cv. Icar				
Control, H_2O	50.6 ± 1.71	54.02 ± 1.22	273.34 ± 10.65	2395.08 ± 10.65
Trifenamid	72.3 ± 1.62	50.28 ± 0.98	363.52 ± 11.27	3377.07 ± 11.27
Dyphesine	69.2 ± 1.46	47.13 ± 1.07	325.15 ± 10.80	2998.44 ± 10.80
Lycopersicon esculentum L., cv. Lider				
Control, H_2O	43.3 ± 1.87	48.99 ± 0.76	212.13 ± 10.18	3051.00 ± 0.18
Trifenamid	52.8 ± 2.36	56.92 ± 1.20	300.54 ± 14.33	4310.85 ± 4.33
Dyphesine	42.6 ± 0.89	55.83 ± 2.02	237.84 ± 7.05	3542.55 ± 7.05

During experiences, it was recorded an increase of cucumber and tomato plant productivity by 32.99 and 44.68%, in the corresponding variants supplemented with Trifenamid, and by 19.24-12.12%, in those treated with Dyphesine. As follows from obtained data, a major effect on plants of *Cucumis sativus* L. and *Lycopersicon esculentum* L. had the treatment with vitamin PP based compounds. Fructification increased by 50-52%, the average fruit weight, detached in the same terms, prevails over control by 15-17% (average weight of cucumbers in variants treated with PhAS was somewhat lower, while the number of fruits higher than in the control variant). Major yields were also obtained at the collection of early production.

Fig. III.7. The influence of sowing seed and foliar apparatus treatment on the growth of cucumber plants.

At the same time it has been established the beneficial influence of water-soluble polymers on plant growth, development and yield (Tab. III.14). Seed treatment before sowing and plant treatment during vegetative growth with Trifenamid preparation, in combination with vinylpyrrolidone / methacrylate of potassium copolymer and / or ammonium, accelerates plant fructification and ensures productivity and yield. As follows from the obtained data, Trifenamid preparation ensured an increase in yields per unit area by 10% as compared to control.

Table III.14. The influence of the combination of Trifenamid preparation with coVP hydro-soluble polymer and M45 Dethane fungicide on productivity and yield structure of *Cucumis sativus* L., cv. Icar plants.

Variants	Fruit number, un pl.$^{-1}$	Average weight of 1 fruit, g	Productivity, g pl.$^{-1}$	Yield, kg/m^2	Efficacy, % control
Control + Dethane M45	2.7 ± 0.07	63.5 ± 1.22	171.7 ± 4.72	1.545 ± 0.03	100.0
Trifenamid+ Dethane M45	2.9 ± 0.08	64.9 ± 0.94	189.2 ± 3.25	1.702 ± 0.05	110.2
Trifenamid+ Dethane M45+coVPNH$_4$K	3.1 ± 0.01	66.6 ± 1.31	205.2 ± 5.17	1.847 ± 0.06	119.6

Being applied in combination with coVPNH$_4$K polymer, the yield supplement constituted 19.6% as compared to control plants, and 8.5% as compared to plants treated only with Trifenamid.

Thus, the exogenous supplement of PhAS, derivatives of vitamin PP, ensures a veridical increase of harvest compared to control plants. The results of determining the elements of forming cucumber and tomato plants productivity reflect their real dependence on the nature of the PhAS used for treatment. A major and authentic effect is obtained at plant treatment with aqueous solution of Trifenamid. Trifenamid conditions the stimulation of germination energy, accelerates root system growth and assimilating apparatus formation, increases productivity and harvest of *Cucumis sativus* L. plants per unit area. The use of Trifenamid in combination with coVP hydrosoluble polymer, ammonium and / or potassium enhances the beneficial effect on plant biological performances, basically by improving water status in plant tissues. It was established compatibility between Trifenamid with Dethane M45 fungicide.

The effect of trinuclear clusters on productivity and yield quality of Arachis hypogeae L.

Peanuts are rated as perspective plants of great economic importance due to their high content of fats (44.9-61.6%) and proteins (25.6-28.2%) in grains. Peanut grains are used in food industry. The other plant organs and waste from the food industry have a major forage value. The crop achieves high and stable production of grain (20.8 to 21.0 q/ha) with a high solids content. Peanuts are megatherm plants, require a minimum of fertilizers, are good at crop rotations and improve less productive soils. Agrobiologic research [111] showed that Moldova’s climatic conditions favor the profitable cultivation of peanuts. However, investigations were undertaken to increase the productivity of these precious plants. In this aspect, a growing interest represent the complex compounds with metals due to their use in agriculture for the regulation of plant growth and productivity, as well as for filling the deficiency of microelement-metals.

During three years of field experiments, it has been studied the impact of hexa-μ-acetate(O,O′)-μ_3-oxo-tris(N,N-diethylnicotinamide)-diiron(III)cobalt(II) monohydrate, conventionally – Difecoden, and hexa-μ-acetate(O,O′)-μ3-oxo-tris(N,N'-diethylnicotinamide)-diiron(III), manganese(II) dihydrate, conventionally – Difemanden, on peanut productivity and yield quality, *Fazenda 2* variety. The scheme of experiences included the following variants: 1 – control, plants treated with water; 2 – plants treated with Difecoden, in the concentration of 0.001% mas, with an optimal effect, established in previous research [98]; 3 – plants treated with Difemanden in the form of aqueous solutions in the concentrations of 0.0001%...0.1% mas. Each variant has been achieved in three repetitions, distributed at random. The treatment was applied twice, on seeds – before sowing, and on the foliar apparatus – during plant flowering.

Data prove the beneficial effect of Difecoden and Difemanden on biological performances of peanut plants of *Fazenda 2*, the differences being in quantity (Tab. III.15).

Table III.15. The influence of treatment with coordination compounds on the formation of elements of peanut plant productivity and yield.

Variants		Number of pods per plant	Plant productivity, $g \cdot pl^{-1}$	Weight of 1000 grains, g	Grain productivity, q/ha	Efficiency compared*, % to control
control, H_2O		14.8 ± 0.5	10.4 ± 0.3	487.9 ± 8.6	21.9 ± 0.6	100
Difecoden, 0.001%		20.4 ± 0.6	11.3 ± 0.3	475.4 ± 9.9	23.8 ± 0.3	117.8
Difemanden	0.0001%	13.2 ± 0.4	9.0 ± 0.4	444.3 ± 12.9	19.0 ± 0.4	86.7
	0.001%	15.3 ± 0.4	11.9 ± 0.5	480.7 ± 13.3	24.5 ± 0.9	109.8
	0.01%	15.1 ± 0.5	11.5 ± 0.3	526.7 ± 12.0	24.1 ± 0.4	107.7
	0.1%	17.10 ± 0.5	10.4 ± 0.3	436.8 ± 11.4	21.9 ± 0.3	104.9

* – effect on pod formation, productivity and plant yield.

Experimental data prove that Difemanden, in the interval of concentrations 0.001-0.1% influences peanut plant productivity, especially grain size. The weight of 1000 grains treated with Difemanden exceeds the weight of 1000 grains of the control sample by 5-10%. Plant treatment with Difecoden ensures yield increase by 8.7%, but reduces grain size by 97.4%, as compared to the control sample. The productivity of plants treated with Difemanden exceeds that of control plants by 10.5-14.5% and by 2.0-5.3% that of plants treated with Difecoden. A major effect was recorded on the plants treated with Difemanden, in the concentration of 0.001-0.01%. It was tested the reproductability effect of coordination compounds on the biological performances of peanut plants. In field experiences the plants were treated (seminally and foliarly) with aqueous solutions of Difecoden – 0.001% and of Difemanden – 0.001% (Tab. III.16). Statistical analysis of data demonstrates the major effect of Difemanden on the elements of peanut plant productivity and yield. The preparation stimulates fructification (number of pods in the plant), conditions the increase of yield per unit area and improves grain trade quality.

Table III.16. The effect of peanut plant treatment (*Arahis hypogaeae* L.) with aqueous solution of Difemanden – 0.001% on productivity and grain absolute weight.

Variants	Number of pods per plant	Plant productivity, g pl^{-1}	Weight of 1000 grains, g	Grain yield, q·ha	Efficiency on pods and yield formation, % as compared to control
Control, H_2O	15.2 ± 0.7	9.4 ± 0.3	487.1 ± 7.2	19.8 ± 0.6	100
Difecoden	20.6 ± 1.11	11.8 ± 0.6	467.1 ± 22.2	24.8 ± 1.2	128.8
Difemanden	20.8 ± 0.9	14.6 ± 0.6	511.4 ± 7.2	29.9 ± 1.4	147.07

The weight of 1000 grains veraciously exceeds the weight of the same number of grains in the control plants and of those treated with Difecoden. Probably, the specific effect of Difecoden and Difemanden on the biological performances of *Arachis hypogaeae* L., plants can be explained through their beneficial reaction at the exogenous supplement of [$Fe^{3+}{}_2$, $Mn^{2+}O$].

Thus, it has been recorded a new regulator of peanut growth – hexa-μ-acetate(O,O′)-μ_3-oxo-tris(N,N′-diethynicotinamide)-diiron(III)manganese(II) dihydrate. The treatment proceeding stipulates the treatment of peanut seeds before sowing and the treatment of leaves during plant flowering with an aqueous solution of hexa hexa-μ-acetate(O,O′)-μ_3-oxo-tris(N,N′-diethynicotinamide)-diiron(III)manganese(II) dihydrate in the concentration of 0.0001-0.1$\%_{mas}$.

The influence of above mentioned bioregulators is reflected in the change of growth rate and in the duration of foliage apparatus activity, without changing the duration of plant vegetation period in general. As a result, there is a veridical increase of overall productivity, the yield being its resultant, plus the number of plants per unit area. Perhaps, an important role in all this belongs to germination energy acceleration and root system rapid development, which were noted at the initial stages of ontogenesis, that condition a better plant nutrition and water supply.

The effectiveness of PhAS depends on genotype, preparation features, dose, mode and period of use. It has been proved the temporary effect of reducing humidity deficiency impact on plant biological performance. Growth processes optimization and the change of organ function duration can be achieved through productivity increase and yield quality characteristics.

The effect of coordination compounds with Co and B on sugar beet productivity (Betta vulgaris L. Saccharifera*).*

Beta is a kind of major economic importance and the main crop providing Moldova with raw material for sugar [112]. More than a quarter of the world's sugar production is produced from the rhizocarpons of this culture. Yet, the yields obtained in most areas of cultivation do not correspond to the potential possibilities of this crop. Yield and sugar production vary greatly in accordance with soil and climatic conditions; in particular, humidity and mineral nutrition elements are the most severe environmental factors affecting agricultural production and its quality [114].

A significant and yet unexploited potential to increase the productivity of agricultural plants is considered the use of physiologically active substances, such as hormonal-type compounds, vitamins, amino acids, antioxidants, microelements etc., whose effects can regulate growth and development, modify various metabolic processes, that intensify and amplify the vegetal organism adaptive properties and the fuller achievement of productivity potential.

In recent years, a special attention is given to compounds with a broad spectrum of activity, that combine PhAS properties, have trophic effects, and are active in low concentrations. It has been established that the amendments with such PhAS reduce pressing on environment by reducing the dosage of chemicals, especially, those of hormonal type used for this purpose. It should be mentioned that most compounds with effects on plant growth and development are imported preparations, whence the need to obtain ecologically innocuous PhAS from less expensive raw material.

Sugar beet is a particularly sensitive plant to microelement deficiency, especially to boron and cobalt [115, 116]. The lack of boron causes the putrefaction of beet „heart”, reduces the rate of sugar transport, a phenomenon particular to dry summers. Currently, it is very actual the question of efficient application of fertilizers aimed at productivity optimization and accumulation of sugar in crop sterns, by minimizing their administration in soil, thus avoiding environmental pollution. Therefore amendments with physiologically active substances, which contain trace elements are beneficial for achieving an optimal production of crop sterns with a maximum sugar output. In this context, it presents a specific interest the perspective of obtaining new physiologically active substances, valuable for sugar beet crop.

The indicated compounds were tested according to standard requirements for PhAS sreening in laboratory experiences, vegetation and on small parcels in the field. In laboratory experiences, it was studied the effect of seed treatment by means of vacuum infiltration and inlay with different doses of Difecoden and Cobamid – coordination compounds containing cobalt and iron, and cobalt and boron at start reactions of plant ontogenesis. As research object served the sugar beet plantlets and plants of Baracuda variety. Field experiences were carried out on small parcels (S=15 m^2) in blocks and have been repeated three times with a randomized division of variants: I-st variant – seeds treated with water (control); II-nd variant – seeds treated with an aqueous solution of Difecoden (0.001%); III-rd variant – seeds treated with an aqueous solution of Cobamid (0.001%). Seed treatment with the above mentioned solutions by volume in a ratio of 2:1, was achieved 4-5 hours before sowing. During the growth period, there were performed two foliar treatments of plants with the mentioned solutions: I-st – at the phase of leaves union in lines; II–nd at the phase of leaves union between lines. Solution consumption per ha is about 200-250 L.

Difecoden – $[C_{42}H_{62}CoFe_2N_6O_{17}]$ represents a complex compound that contains iron and cobalt, attibuted to the group of trinuclear clusters with properties of PhAS [98]. The Cobamid – $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$ is a coordination compound of cobalt(III) and boron, on the basis of dimethylglyoxime (DH_2) and nicotinamide (PP) in the form of a brown crystalline substance, soluble in water, methanol and ethanol, and insoluble in diethyl ether [95]. The complex is part of cobalamin class and can be considered the simplest model of the active center of vitamine B_{12} molecule. Nicotinamide of both coordination compounds composition is a substance with a regulating and energetic role, widely met in vegetal organisms. It is a functional part of pyridine nucleotide – oxidative equivalents (NAD^+) in the catabolic processes, or reducers (NADR) in anabolic processes.

Within IR spectrum of $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$ complex can be noticed the presence of the following absorption bands: $\nu_{as}(NH_2)=3359$, $\nu_s(NH_2)=3160$, $\nu(C=O)=1692$, $\delta(NH_2)=1637$, $\nu(C=C)+\delta(CCH)=1609$, $\nu(C=N)=1562$, $\delta_{as}(CH_3)=1444$, $\nu(N=O)=1097$, $\delta(CCH)=1167$, $\rho(CH)=697$, $\nu(Co\text{-}N)_{DH}=515$, 429, $\nu(Co\text{-}N)_{PP}=459$ cm^{-1}. The presence of $[BF_4]^-$ anion within the complex is confirmed by $\nu_{as}(BF_4)=1085$ cm^{-1}, $\nu_s(BF_4)=750$ cm^{-1}, $\delta(F\text{-}B\text{-}F)=545$ cm^{-1} absorption bands.

Within $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$ it performs a structure of ionic type containing the $[Co(DH)_2(Nia)_2]^+$ cations, $[BF_4]^-$ anions and molecules of crystallization water (Fig. III.8).

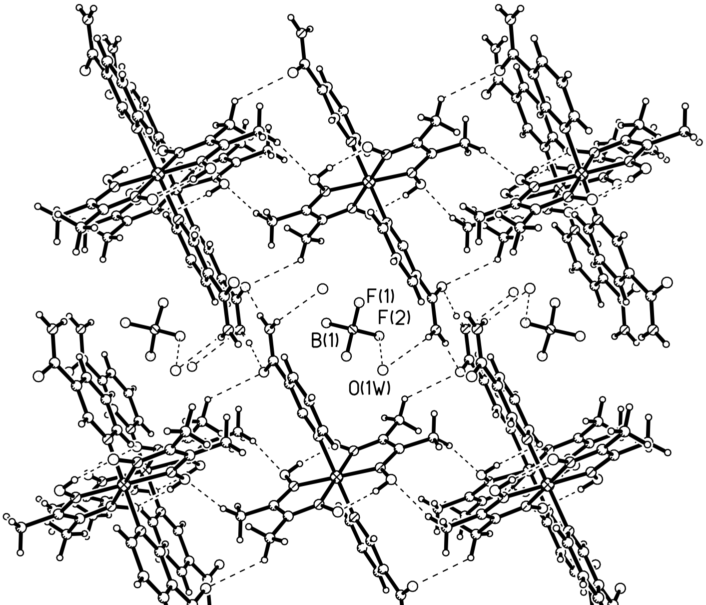

Fig. III.8. Fragment of crystalline structure in $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$.

Octahedral complex cations are stabilized in the equatorial plane by intramolecular hydrogen bonds O–H···O, joining two monoanions of dimethylglyoxime in a stable pseudo macrocyclic system. Apical ligands – nicotinamide coordinates to the complex generator via the nitrogen atom of the aromatic heterocycle [116].

The neighbouring $[Co(DH)_2(Nia)_2]^+$ cations are joined by N-H···O hydrogen bonds, forming chains. Within the formed canals are located the $[BF_4]^-$ anions and water molecules joined among them by hydrogen bonds (Fig. III.9).

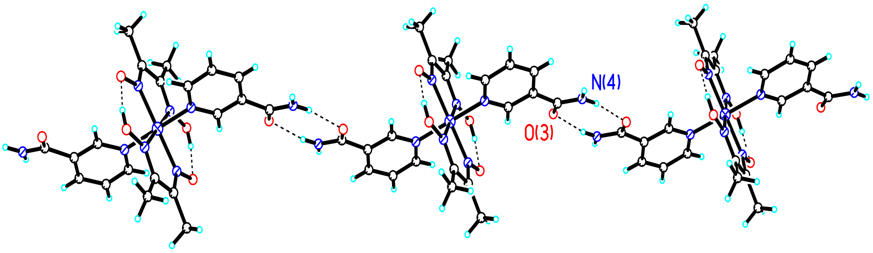

Fig. III.9. Hydrogen bonds and unidimensional pseudo chain formation in $[Co(DH)_2(Nia)_2][BF_4]\cdot 2H_2O$.

It should be known that microelements, due to their cathalitic features, favor in plants the efficient use of water, nutrients and solar energy, fact positively effecting productivity and yield quality. It became an axiom the finding that some microelements, particularly iron, cobalt and boron, participate in the processes of carbon assimilation, respiration, enter in the composition of enzymes responsible for the synthesis of chlorophyll pigments, enhance the activity of dehydrogenases, nitrate reductase etc. Cobalt influences the structure and functional state of the photosynthetic apparatus, contributes to pigment background increase, stimulates growth processes due to the change of phytohormones balance in favor of auxin, participates in oxydo-reduction reactions, and in the synthesis of nucleic acids [115].

Boron deficiency conditions the disorder of carbohydrate synthesis, conversion and transfer [114]. Based on these findings it was logical to expect that nicotinamide coordination compounds with Co and Fe and, in particular, with Co and B would have a positive impact on the biological performances of sugar beet plants.

The results of this investigation prove the beneficial effect of coordination compounds with Co^{2+} and Fe^{3+}, in particular, with Co and B, on the growth and development of *Betta vulgari*s L. plants at the initial stages of ontogenesis (Fig. III.10 and III.11; Tab. III.17).

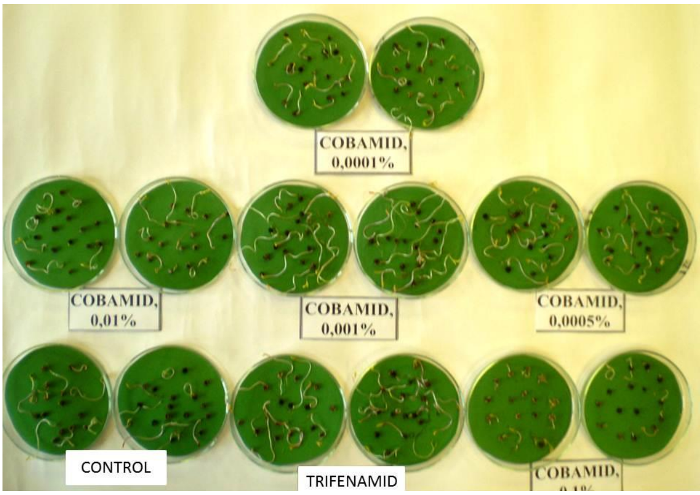

Fig. III.10. The effect of sugar beet treatment on germination and seedlings growth rate at the initial stage of ontogenesis (9 days after treatment).

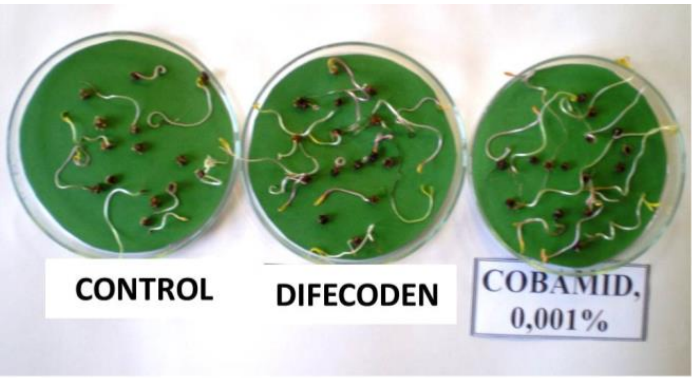

Fig. III.11. The effect of sugar beet treatment with physiologically active substances on seedlings growth and development at the initial stage of ontogenesis (9 days after treatment).

It has been noted an acceleration in germination and plantlet growth under the influence of studied compounds, fact that indirectly proves their effect on cell division and cytoplasm component biosynthesis. The effect of seed treatment with Difecoden, 7 days after germination, consists in stimulating plant weight by 90% as compared to the plantlets of control sample. A major influence ensures the treatment of seeds with Cobamid solution in a concentration of 0.001 and 0.0005%. In these variants, it was observed the intensification of weight accumulation by 154.5 and 90.9%, as compared to plantlets from control variants. The range of effective concentrations of Cobamid solutions is of 0.0001-0.001%.

Table III.17. The influence of PhAS on seed germination energy and growth of cv. Baracuda sugar beet variety at the initial stage of ontogenesis (7 days after germination).

Variants	Germination energy,%		Seedlings weight, g/pl		Efficiency, % control
	M ± m	Δ, %	M ± m	Δ, %	
Control	76.67 ± 1.12		0.33 ± 0.003		100
Difecoden, 0.001%	80.00 ± 1.08	+4.34	0.42 ± 0.005	+90.0	104.6
Cobamid, 0.1%	53.34 ± 0.47	-30.43	0.14 ± 0.002	-36.36	69.89
Cobamid, 0.01%	56.67 ± 0.52	-26.08	0.23 ± 0.004	+4.54	74.00
Cobamid, 0.001%	83.33 ± 0.98	+8.68	0.56 ± 0.009	+154.5	109.10
Cobamid, 0.0005%	83.33 ± 2.01	+8.68	0.42 ± 0.007	+90.0	108.9
Cobamid, 0.0001%	80.00 ± 1.27	+4.34	0.31 ± 0.004	+40.91	104.45

The administration of coordination compounds with Co and Fe, Co and B by treating seeds and foliar area favored the formation of an assimilating apparatus with a high content of pigments (Tab. III.18).

Thus, Difecoden ensured an increase in chlorophyll and carotenoids content by 10.10 and 5.51% respectively, as compared to control variant plants. The plants treated with Cobamid were characterized by an assimilating apparatus with a high pigment content, as compared to the plants treated with Difecoden solution, and to control plants. The obtained data demonstrate the higher efficacy of Cobamid as compared to Difecoden.

Table III.18. The influence of plant treatment with Difecoden and Cobamid solutions on pigment content (mg/100 g m.p.) from the leaves of sugar beet plants.

Variants	Chlorophyll *a*		Chlorophyll *b*		$\sum a + b$		Carotinoids	
	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control
Control	113.1±0.2		51.9±0.2		165.0±0.4		37.2±0.2	
Difecoden, 0.001%	127.5±1.2	12.76	54.2±1.2	4.29	181.7±2.4	10.10	39.3±1.7	5.51
Cobamid, 0.001%	138.8±0.3	22.72	66.9±0.3	28.79	205.6±0.6	24.63	54.1±0.3	45.44

It should be mentioned that the dynamics and intensity of physiological peocesses taking place in plants during ontogenesis by virtue of „cause-effect” link, inevitably influence plant growth and productivity. The PhAS amendments truthfully influenced plant productivity (Tab. III.19). Difecoden conditioned an increase of production by 5.1%, while the application of cobamid generated a yield supplement of 11.5% as compared to the quantity of control plant yield. Comparative analysis of data presented in Table III.19 allows us to assume that the plants of *Betta vulgaris* L., treated with physiologically active substances with cobalt and iron, cobalt and boron, can maintain metabolic processes at a higher level, unlike control plants, which affects plant productivity and production quality.

This conclusion is confirmed additionally by the results of determining sugar content in rhizocarpons (Tab. III.19). The treatment of sowing seeds and plants during vegetation with Cobamid increased sugar production by 18.4% as compared to control sample, and by 6.6% as compared to plants treated with Difecoden. Cobamid effect on productivity and sugar content is significantly higher than that of Difecoden.

Table III.19. The influence of PhAS on plant yield and sugar content in rhizocarpons.

Variants	Yield				Sugar content in rhizocarpons, %		Total sugar content, t/ha	
	On parcel, kg/15 m^2		t/ha					
	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control
Control	54.2±0.7		37.4±0.5		19.2±0.6		5.6±0.2	
Difecoden, 0.001%	57.0±0.9	5.2	39.3±0.7	5.1	20.9±0.7	7.21	6.1±0.2	13.1
Cobamid, 0.001%	60.5±0.9	11.6	41.7±0.6	11.5	20.7±0.4	5.67	6.5±0.1	18.4

Hence, plant treatment with aqueous solutions of Difecoden and Cobamid coordination compounds, ensures the increase of rhizocarpon yield and sugar content, as compared to control plants, fact that, consequently, contributes to sugar production increase per unit area.

On the basis of obtained data, we can conclude that coordination compounds with cobalt and iron, cobalt and boron are physiologically active substances with a positive impact on the productivity and production quality of sugar beet plants. A maximum effect can be obtained at the treatment of sugar beet seeds before sowing and of plant foliar apparatus with aqueous solutions of Cobamid.

The influence of PhAS on the growth and productivity of beet plants (Béta vulgáris L. var. *conditiva Alef.*).

As we have mentioned above, the development and implementation of tolerance inducing, impact attenuating and plant productivity stabilization methods open great perspectives for the agriculture of countries located in zones of climatic risks.

A special attention is, presently, given, all over the world, to compounds with a broad-spectrum activity, combining PhAS properties, displaying trophic effects, and active in small concentrations. In a series of laboratory experiences it has been studied the effect of seed treatment before sowing and plant treatment during vegetative growth – at the stages of „leaves union in lines" and „leaves union between lines" with aqueous solutions of composite preparation on the growth and development of sugar beet plants of Cilindric and Egipteana plată varieties [97]. The composite preparation represents a mixture of subsatnces having at their basis some of the most important microelements and vitamins: iron, cobalt zinc, manganese, boron, nicotinamide (vitamin PP), and nitrate anion. Iron and cobalt were included in the form of coordination compounds, while zinc and manganese – in the form of nitric acid salts. Seed treatment was achieved through imbibation during 2 hours with the corresponding solutions of the same concentration, in a ratio of 1:1, seed:solution. In order to determine the physiologically optimal concentration of the Composite preparation, it was investigated the effect of solutions in the concentrations of 0.00005-0.1% mas. on plant growth at the initial stages of ontogenesis. The obtained results show that the Composite preparation is physiologically active, the major effect being obtained at the use of solutions in concentrations of 0.0001-0.0005% (Fig. III.12).

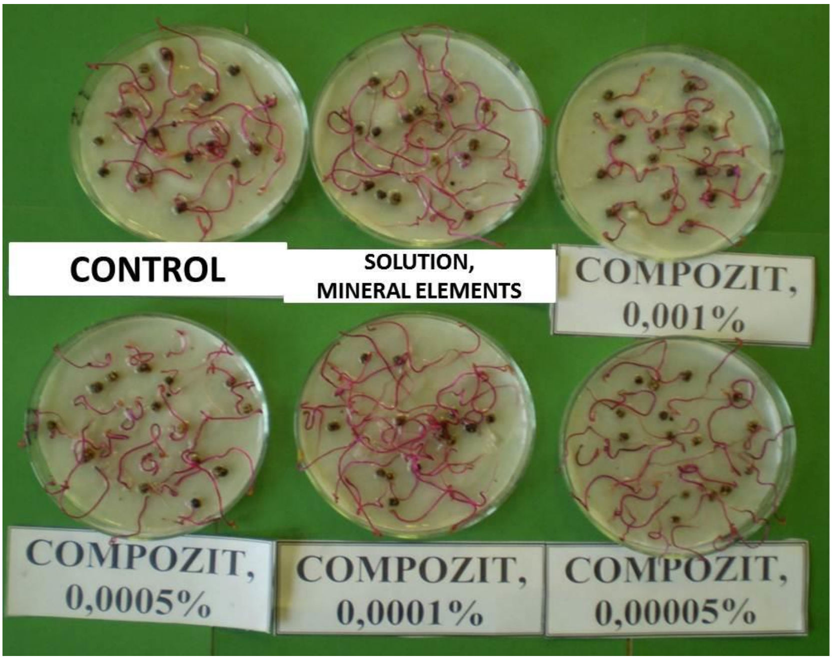

Fig. III.12. The effect of seed treatment with PhAS on beet plants (cv. Cilindrica) development at the initial stage of ontogenesis.

Seed treatment with such solutions ensures the intensification of plant growth as compared to the growth of both control plants and plants from seeds treated with solutions containing corresponding elements in the form of mineral salts (Tab. III.20; Fig. III.13).

It has been found that seed treatment with Conimid solution of 0.0001% increases germination energy by 38.1% as compared to control variant, and by 26.1% as compared to germination of seeds treated with mineral salt solution of the same elements. Plantlet growth processes at the initial stage of ontogenesis significantly intensify under the influence of coordination compounds: by 74.0% as compared to plantlet growth in the control variant, and by 13.9% as compared to the growth of plantlets from seeds treated with mineral salt solution.

Table III.20. The influence of PhAS on seed germination energy and beet (cv. *Egipteană plată* variety) plantlet growth at the initial stage of ontogenesis (7 days after germination).

Variants	Germination energy, %		Plantlet weight, mg/pl		1 plant weight, mg	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	70.0±1.49		342.33±6.65		24.45±0.48	
Solution, mineral elements	**76.66±1.51**	**9.51**	**523.04±2.11**	**52.80**	**37.36±0.62**	**52.80**
Composite, 0.00005%	100.00±00	42.86	540.33±6.79	20.41	29.45±0.65	20.41
Composite, 0.0001%	**96.66±1.50**	**38.09**	**597.70±7.45**	**74.03**	**42.55±0.60**	**74.03**
Composite, 0.0005%	93.33±1.48	33.33	471.10±11.9	37.63	33.65±0.55	37.63
Composite, 0.001%	93.33±1.49	33.33	332.50±1.09	2.86	23.75±0.69	2.86
Composite, 0.02%	86.66±2.98	23.80	308.70±3.75	-9.82	22.05±0.54	-9.82
Composite, 0.1%	80.0±2.98	14.29	177.38±2.95	-48.18	12.67±0.37	-48.18

In field experiences, it has been established that the plants treated with Composite preparation solution are characterized by a higher content of assimilating pigments (Tab. III.21), intensification of carbon assimilation, transpiration, water use efficiency, and by a better growth and development (Fig. III.13), unlike plants treated with solutions of mineral salts of the same elements, and plants of the control variant.

Table III.21. The content of assimilating pigments (mg 100 g^{-1} m. p.) in leaves of beet plants, Cilindrica varety.

Variants	Chlorophyll *a*, mg/100 g w.f.		Chlorophyll *b*, mg/100 g w.f.		Carotinoids, mg/100 g w.f.		Cl *a*+Cl *b*, mg/100 g w.f.	
	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control
Control	48.0±0.4		11.7±0.4		12.79±0.3		59.74±0.2	
Solution, mineral elements	55.8±0.9	+16.3	20.2±0.3	+71.1	17.61±0.6	+37.7	76.01±0.9	+27.2
Composite	91.4±0.8	+90.5	35.6±0.4	+202.5	28.04±0.7	+119.2	127.0±0.8	+112.6

Fig. III.13. The effect of treatment with PhAS on beet plant (cv. Cilindrica) development.
Legend: 1 – control; 2 – mineral elements; 3 – Composite.

Photosynthesis intensity and water use efficiency in plants treated with the new preparation are essentially higher (Tab. III.22). Photosynthesis intensity and the efficiency of water use by plants treated according to invention are higher as compared to vital processes taking place in plants treated with mineral salt solution and, particularly, to those of control plants.

Table III.22. The influence of plant treatment with PhAS on photosynthesis intensity, transpiration and stomata conductibility, cv. *Egipteană plată.*

Variants	Photosynthesis intensity, Mm/m^2/h	Transpiration intensity, Mm/m^2/h	Water use efficiency	Stomata conductibility, Mm/m^2/h
Control	10.95±0.28	3.55±0.12	3.08±0.09	0.39±0.009
Solution, mineral elements	11.38±0.31	3.75±0.09	3.03±0.07	0.64±0.007
Composite	14.14±0.49	4.02±0.10	3.52±0.08	0.85±0.008

Plant extra radicular fertilization with Composite preparation has a positive impact on plant productivity and production quality. In plants treated with Composite aqueous solutions prevail „big” and „average” fractions of rhizocarpons (Tab. III.23; Fig. III.14).

Table III.23. The effect of PhAS on the yield of beet plants.

Variants	Yield, kg/parcel	Roots, nr	Average weight, g/root	Yield, kg/m^2	% control
Egipteană plată variety					
Control	29.90±0.37	49.0±0.49	610.00±8.11	3.99±0.09	
Solution, mineral elements	38.35±0.44	74.0±0.51	518.24±9.21	4.79±0.12	+20.05
Composite	46.16±0.51	72.0±0.61	641.10±7.21	5.77±0.11	+44.61
Cilindrica variety					
Control	19.862±0.38	63±0.53	312.73±5.14	6.62±0.19	
Solution, mineral elements	23.875±0.53	61±0.44	391.39±4.23	7.96±0.21	+20.24
Composite	29.907±0.44	61±0.38	490.28±4.32	9.69±0.32	+46.37

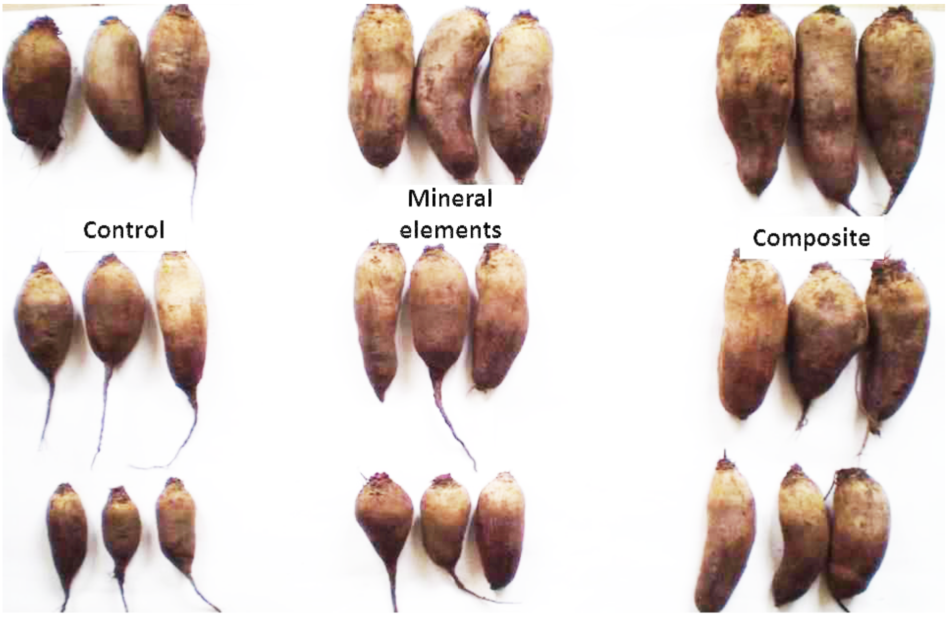

Fig. III.14. The influence of PhAS on morphological characteristics of beet plant rhizocarpons, cv. Cilindrica.

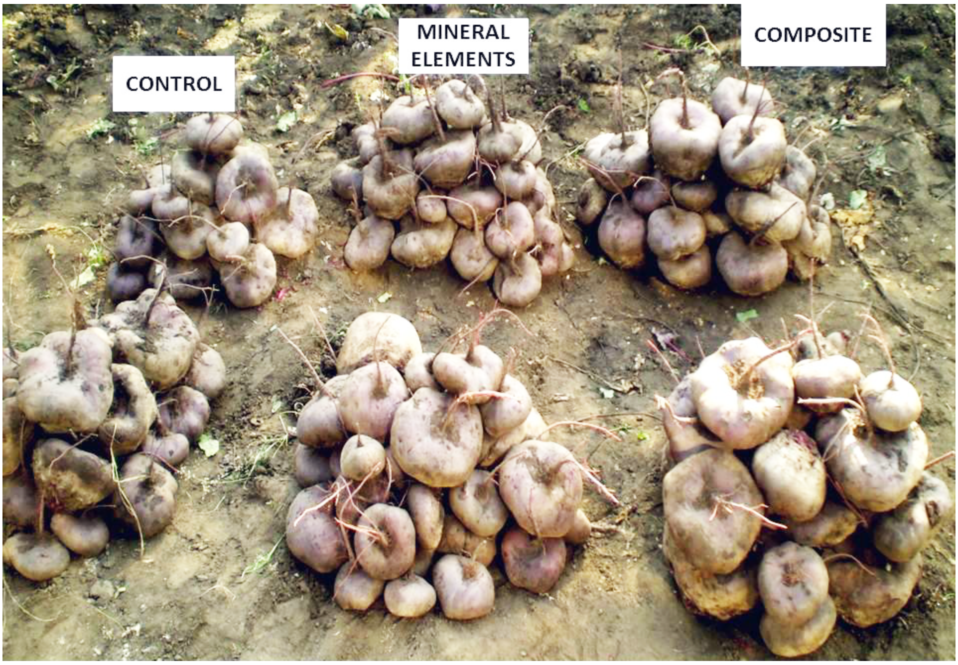

Fig. III.15. The influence of PhAS on morphological characteristics of beet plant rhizocarpons, cv. Egipteană plată.

In conclusion, we underline that Composite preparation possesses phisiologically active features with a positive impact on beet plants productivity and production quality. A maximum effect is obtained at seed treatment before sowing and at plant foliar apparatus treatment with Composite aqueous solutions.

The Physiological effect of Composite preparation+thiourea on plants biological performances in drought conditions: optimization of water use efficiency.

In conditions of acute water deficiency, a significant importance plays the application of agricultural technologies ensuring water saving, including the cultivation of drought resisting plant varieties. In this context the problem of increasing water use efficiency (WUE) in the process of yield growth and formation has an exclusive importance. The processes ensuring the survival of plants in conditions of humidity deficiency are associated with water consumption diminuation through the reduction of evaporation surface as a result of stomata closing and growth inhibition, that lead to productivity decrease. Stomata closing reduces water loss through transpiration, but inevitably leads to photosynthesis diminuation as a result of chlorophyll content reduction and carbon dioxide access at chloroplast level [38, 117]. It is considered that WUE in limited conditions of humidity is a crucial factor for productivity [118-120]. Moreover, WUE is estimated as a component of drought tolerance in natural systems, being considered as an alternating way of its increase [1].

That is why, a better WUE by plants can be obtained through: soil fertility increase, antitranspirants application, cultivation of varieties with a high resisting potential, etc. A widely accepted modality of increasing resistence to drought is considered the possibility of increasing WUE by applying physiologically active substances. Considering this, the research objective consisted in evaluating thiourea effect in combination with Composite preparation containing microelements like: iron, cobalt, zinc, manganese, boron, vitamin PP; iron, cobalt and boron being included in the form of biologically active coordination compounds-on the intensity of photosynthesis, transpiration and plant productivity in conditions of moderate water deficiency.

Investigation results showed significant differences of the reaction of *Zea mays* L. plants, pre-treated with PhAS, at water provision fluctuation and humidity deficiency (Tab. III.24). In optimal conditions of humidity, water content in plant leaves is considerably higher as compared to hydration degree of plants exposed to drought action and reaches values of 77.23±1.28 g water in 100 g m.p. The plants pre-treated with PhAS showed a slight, but statistically veracious trend of increasing hydration degree in leaves. The change of soil humidity and water provision worsening caused water content reduction, turgor diminuation and saturation deficit increase.

In conditions of humidity deficit, hydration degree of control plants reduces by 7.7%, while that of pre-treated plants reduces by about 3.3-5.0%. Saturation deficit in the leaves of plants pre-treated with cytokinins (CK), thiourea (Thio) and Thio+Composite is comparatively more reduced even in optimal conditions of humidity (Tab. III.24).

Table III.24. The influence of PhAS and drought on the parameters of water status in corn plants.

Variants	CA, g/100 g. m. p.		DS, % since full saturation	
	M±m	Δ, %	M±m	Δ, %
70% TWC				
Control	77.23±1.28		7.88±0.22	
Cytokinins	78.20±1.55	+1.25	6.43±0.12	-18.40
Thiourea	78.41±1.78	+1.53	4.52±0.11	-42.64
Thiourea + Composite	78.23±1.57	+1.29	6.38±0.07	-19.04
30% TWC				
Control	71.32±1.48	-7.65	25.42±0.36	222.59
Cytokinins	73.39±1.13	-4.97	24.86±0.28	215.48
Thiourea	73.51±1.09	-4.82	22.53±0.44	185.91
Thiourea + Composite	74.66±1.11	-3.33	20.34±0.37	158.12

At the decrease of soil humidity from 70% TWC to 30% TWC, the value of saturation deficit in the leaves of corn plants of control variant increases from 7.88 to 25.42% out of full saturation of tissues. In the leaves of plants pre-treated with CK, saturation deficit (SD) increases in such conditions from 6.43 to 0.434%. A major effect of stabilizing hydration degree and minimizing drought effect on saturation deficit was noted in corn plants pre-treated with Thio, especially with Thio+Composite. The degree of saturation deficit modification in these plants is significantly smaller in drought conditions (Tab. III.24). Experimental data proved that seed pre-treatment with Thio and Thio+Composite ensured water status optimization in the plants exposed to drought. In drought conditions, in plant tissues it forms a saturation deficit that can be deduced from the relationship between water absorption from soil, its transportation to organs and consumption in the process of transpiration. The considerable growth of SD in leaves can be caused by an increased consumption of water or by the diminuation of its absorption by roots, fact that indicates on the disturbance of plant hydric balance.

It was established that thiourea, especially thiourea in combination with Composite preparation, has a significant positive influence on key physiological processes correlated with plant productivity (Tab. III.25).

The use of pre-treated seeds for sowing and plant treatment during vegetative growth conditioned a veracious increase of assimilating pigments content.

The effect of plant treatment with Thio+Composite exceeds the effect of plant treatment with Thio by 14.4%.

Table III.25. The influence of PhAS and drought on pigments content in corn plant leaves.

Variants	Chlorophyll *a*, mg/100 g. m. p.	Chlorophyll *b*, mg/100 g. m. p.	Chlorophyll *a*+*b*, mg/100 g. m. p.	Carotinoids, mg/100 g m. p.
	M ± m	M ± m	M ± m	M ± m
70% TWC				
Control	140.5±2.2	59.9±1.1	200.5±2.9	33.6±0.9
CK	168.4±2.3	71.1±1.2	239.5±3.6	44.9±0.7
Thio	180.73±0.6	68.62±1.9	249.35±3.1	42.77±0.5
Thio+Composite	213.14±0.4	80.77±2.1	293.91±3.2	47.33±0.7
30% TWC				
Control	137.14±0.8	54.41±0.5	191.55±1.2	31.10±0.6
CK	153.10±0.6	68.60±0.5	220.87±2.3	39.37±0.5
Thio	156.81±0.5	65.33±0.9	222.14±1.6	40.45±0.2
Thio+Composite	175.5±0.8	67.83±0.7	243.33±2.5	44.39±0.4

One of the early effects of drought advancement and water deficiency in the soil is the reduction of leaves growth, the diminuation of solar radiation interception and, subsequently, the reduction of dry material production. The analysis of data obtained by using the portable gas analyzer LCA-4 in experiences achieved in similar conditions of humidity and temperature revealed the beneficial effect of substances used for treatment on WUE of *Zea mays* L. plants (Tab. III.26).

Table III.26. The influence of drought on WUE of corn plants.

Variants, soil humidity, % TWC	Stomata conductibility Mm/m^2/h	Transpiration intensity, Mm/m^2/h	Photosynthesis Intensity, Mm/m^2/h	WUE Mm CO_2/Mm H_2O
IX-th leaf phase, VII-th drought day				
70	0.02	2.25	11.52	5.12
30	0.003	1.08	3.13	2.90
Flowering phase, VII-th drought day				
70	0.1	2.64	9.86	3.73
30	0.04	0.77	1.95	2.53

At flowering phase, when air humidity significantly decreased, while temperature and solar radiation significantly increased, the process of carbon dioxide assimilation diminished even in plants on a background of favorable humidity (70% TWC). Their photosynthesis intensity constituted 85.6% out of the process intensity recorded at the „IX-th leaf stage". In this period, humidity deficiency caused the inhibition of stomata conductibility by 60.0%, transpiration by 70.8%, carbon assimilation by 80.2%, as compared to the corresponding parameters of control plants. Within plants pre-treated with PhAS, as an optimization consequence, water status, assimilation process, WUE and, as a result, the reproductive process maintain at a higher level (Tab. III.27).

Table III.27. The influece of PhAS and drought on assimilation intensity of corn and soy plants.

Variants	Photosynthesis intensity, mM/m²/sec		Transpiration intensity, mM/m²/sec		WUE mM CO_2/mM H_2O	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Glycine max , cv. Enigma						
Control	8.65±0.24		4.01±0.11		2.16±0.05	
CK	14.95±0.37	72.83	4.90±0.09	22.19	3.05±0.09	41.20
Thio	15.95±0.45	84.39	5.18±0.14	29.18	3.08±0.08	42.59
Thio+Composite	22.77±0.61	163.24	5.82±0.12	45.13	3.91±0.08	81.02
Zea mays, cv. P 459						
Control	24.25±0.56		3.92±0.02		6.18±0.07	
CK	26.10±0.45	7.63	4.12±0.06	5.10	6.33±0.04	2.43
Thio	27.31±0.48	12.62	4.28±0.04	9.18	6.38±0.05	3.24
Thio+Composite	31.4±0.21	29.45	4.49±0.05	14.54	6.99±0.07	13.11

The dynamics and intensity of physiological processes taking place in plants during ontogenesis in drought conditions inevitably influence plant growth and development (Tab. III.28). Plant pre-treatment with Thio, especially with the mixture of Thio+Composite, significantly reduced the impact of hydric stress on foliar apparatus formation.

Foliar area reduction represents the main cause of grain yield decrease, because the development of foliar area determines dry substance accumulation during flowering and grain filling, influences yield index through its effect of balancing water use before and after flowering. As compared to control plants, plant treatment reduced drought impact on biological performances, thus ensuring a better productivity.

Table III.28. The effect of PhAS on biological performances of corn plants in drought conditions.

Variants	Height, dm		Foliar area, dm^2		Productivity, g/pl.	
	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %
In conditions of optimal humidity						
Control	12.2±0.3		37.8±0.4		52.8 ± 0.5	
CK	14.3±0.1	+17.5	39.0±0.3	+3.2	61.7±0.4	+16.8
Thio	16.1±0.4	+32.5	39.2±0.2	+3.6	69.3±0.6	+31.3
Thio+ Composite	13.0±0.2	+7.2	39.3±0.4	+4.0	70.0± 0.9	+32.6
In drought conditions						
Control	9.8±0.1	-18.9	30.2±0.3	-20.1	34.8±0.5	-39.0
CK	11.2±0.1	-7.8	32.3±0.3	-14.6	38.9± 0.3	-26.2
Thio	10.7±0.2	-11.2	34.0±0.4	-10.2	37.7± 0.6	-28.6
Thio+ Composite	12.2±0.2	+0.7	35.7±0.3	-5.6	39.7±0.9	+24.7

Drought influence on plants treated with thiourea was 1.5 times smaller while on those treated with Thiourea + Composite was 2.14 times smaller, as compared to the impact on untreated plants. Therefore, the treated plants have a higher capacity to regulate water status manifested by the increase of plant tissues capacity to retain water through its efficient use, by enabling biosynthesis and phytomass accumulation, which, ultimately, ensure the formation of more vigorous plants and reduce yield losses in suboptimal conditions of humidity.

Exogenous application of PhAS, particularly of thiourea in combination with Composite, conditions the increase of gas exchange, stomata conductance, photosynthesis, transpiration, and therefore WUE. The effect of plant pre-treatment with thiourea and Thio+Composite combination on the content of assimilating pigments, carotenoids, in dry conditions exceeds that produced by cytockinins. The administration of physiologically active compounds by optimizing water status, the content of assimilating pigments and photosynthesis process had a positive impact on plant growth, development and productivity.

The influence of coordination compounds on biological performances of ***Phaseolus vulgaris L.*** *plants.*

It is well known the proceeding of cultivating plants by treating seeds before sowing and foliar apparatus with a 0.001% mas. aqueous solution of biologically active substance – heteroauxin (IAA). The treatment has a beneficial influence on plant productivity and yield. In a series of laboratory experiments and vegetative conditions it has been studied the effect of treating seeds before sowing and plant during vegetative growth at „bud-flowering” and „pod growth” phases with IAA solution and Conimid solution on the growth and development of bean plant. The essence of the new process comprises the treatment of seeds by imbibation for 2 hours, in a ratio of 1:1, seed: solution, and plant treatment during vegetative growth – at bud-flowering phase and at pod growth phase – with aqueous solution of biologically active substance nitrate of *trans*-bis(dimethylglyoximato)bis(nicotinamide)cobalt(III) dihydrate – conventionally Conimid (Tab. III.29).

The plants treated with Conimid show intensified processes of carbon assimilation, transpiration and water use efficiency, unlike plants treated with IAA, especially with control plants.

Table III.29. The effect of PhAS on photosynthesis intensity, transpiration, stomatal conductibility of leaves and water use efficiency (WUE) of *Phaseolus vulgaris* L. plants in drought conditions of 2012.

Variants	Photosynthesis intensity, Mm/m^2/h	Transpiration intensity, Mm/m^2/h	WUE,* MmCO$_2$/MmH$_2$O	Stomatal conductibility, Mm/m^2/h
Control	3.59 ± 0.09	1.31 ± 0.009	2.74 ± 0.08	0.015 ± 0.0002
IAA	4.35 ± 0.08	1.53 ± 0.006	2.84 ±0.06	0.018 ± 0.0004
Conimid	5.04 ± 0.07	1.70 ± 0.005	2.96 ± 0.07	0.020 ± 0.0008

* WUE – water use efficiency

The obtained results prove that the processes of carbon assimilation and efficiency of water use by plants treated with Conimid aqueous solution are veridically more intense as compared to the plants treated with solution of indolyl acetic acid (IAA). Phototosynthesis intensity grows by ≈ 21.2% under heteroauxin influence; and by 40.4% under Conimid influence, as compared to control plants. The treatment with IAA ensured a more efficient water use (by 3.75%) by plants for creating a production unit, as compared to control. As for the plants treated with Conimid, water use efficiency increased by 8.03%. The use of physiologically active substances had a positive impact on plant productivity (Tab. III.30).

Plant treatment with Conimid solutions ensured the increase of production by 28.76% in conditions of favorable humidity, and by 30.37% – in drought conditions, as compared to control plants. In comparison to the plants treated with IAA solution, production increase constituted 11.7 and 9.4%, in conditions of favorable humidity and in drought conditions.

At the same time, in the plants treated with Conimid, photosynthesis intensifies and water efficiency increases. Hence, the plants treated with

Conimid aqueous solution, as compared to its closest equivalent, show a better growth and productivity in both optimal and deficient conditions of humidity.

Table III.30. Productivity indices of *Phaseolus vulgaris* L. plants treated with PhAS.

Variants	Productivity, g/plant		Number of grains, pieces/plant		Weight of 1000 grains, g	
	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %
Control	9.11±0.31* 6.42±0.20**		57.85±1.21 40.67±1.02		162.33±4.1 157.86±3.1	
IAA	10.50±0.39 7.65±0.19	+15.26 +19.15	60.48±1.17 46.27±1.08	+4.56 +13.77	173.62±2.1 165.32±2.2	+6.95 +4.73
Conimid	11.73±0.39 8.37±0.21	+28.76 +30.37	64.93±1.12 49.16±1.98	+12.24 +20.87	180.65±4.0 170.25±3.0	+11.29 +7.85

* – indices of plants on optimal humidity backgroud;
** – indices of plants on a humidity deficiency background.

Nitrate of trans-bis(dimethylglyoxime)bis(nicotinamide)cobalt(III) dihydrate – substance with antioxidant properties.

It was proposed the assortment extension of biologically active substances with antioxidant properties and the protection of cell structure against the distructions caused by oxygen reactive species (ORS). It is known that the coordination compound hexa-μ-acetate(O,O´)-μ_3-oxo-tris (N,N´-diethylnicotinamide)-difier(III)cobalt(II) monohydrate – conventionally called Difecoden, has the capacity to regulate plant growth, development and productivity [98]. Difecoden is part of bioactive coordination compounds, but its antioxidant properties are significantly weaker as compared to Conimid.

In a series of laboratory and vegetation experiences it has been studied the effect of plant treatment with Conimid on the indices that characterize the intensity of oxidative destructions (the content of malonic dialdehyde – MDA), the activity of antioxidative protection enzymes (dismutase superoxide – DSO, catalase – CAT, ascorbate peroxidase –APX) and the content of assimilating pigments in plant leaves.The experiences were carried out according to the following scheme: I-st variant – plants from seeds treated with water (control); II-nd variant – plants from seeds treated with Difecoden aqueous solution of 0.001%; III-rd variant – plants from seeds treated with Conimid aqueous solution of 0.001%. The results are exposed in table III.31.

Table III.31. Conimid influence on the content of malonic dialdehyde and on the activity of antioxidative protection enzymes.

Variants	MDA content, mkM• g^{-1} f.w.		SOD, un. conv. • g^{-1} f.w.		APX, mM• g^{-1} f.w.		CAT, mM• g^{-1} f.w.	
	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %	M±m	Δ, %
Control	15.91±0.8		145.74±2.2		3.11±0.1		2.10±0.05	
Difecoden	12.44±0.6	-21.8	154.54±1.9	6.0	4.73±0.2	52.1	3.06±0.06	45.71
Conimid	10.68±0.4	-32.8	165.58±0.9	13.6	5.03±0.3	61.7	3.12±0.09	48.6

The obtained data show that Conimid has a significantly stronger antioxidative effect as compared to Difecoden. Seeds and foliar apparatus treatment with Conimid reduces the formation of malonic dialdehyde – the final product of phospholipids oxidative destruction – by 32.87%, as compared to MDA content in control plants leaves, and by 14.15%, as compared to the plants treated with Difecoden. Potentating the capacity of plant antioxidating protection ensures the stabilization of assimilating pigment complex and reduces clorophyll oxidative distruction (Tab. III.32).

Table III.32. The effect of antioxidative substances on the content of assimilating pigments ($mg \cdot dm^{-2}$) in plant leaves.

Variants	Chlorophyll *a*		Chlorophyll *b*		Carotinoids		Cl *a*+Cl *b*	
	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control	M±m	Δ, % control
Control	1.44± 0.002		0.60± 0.003		0.42± 0.001		2.09± 0.002	
Difecoden	1.91± 0.002	32.6	0.90± 0.003	38.5	0.62± 0.001	47.6	2.80± 0.001	34.0
Conimid	2.32± 0.001	61.8	1.04± 0.005	59.7	0.71± 0.001	70.1	3.37± 0.001	61.2

The plants treated with Conimid are characterized by an assimilating apparatus with a significantly higher content of pigments, unlike the plants treated with Difecoden, as compared to control plants. Thus, Conimid coordination compound shows significantly better expressed antioxidating features than Difecoden.

Elucidating the antioxidating features of ionic type coordination compound $[Co(DH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$.

Nowadays, one of the most important objectives of modern agriculture is to attain production with an increased content of microelements and vitamins protecting against oxygen reactive species, that cause oxidative stress in plants, animals and humans. A special attention is given to products rich in selenium, due to its importance in the food chain. Currently, selenium was identified as an essential mineral nutrient for the human body, with an important physiological role in preventing and even treating some diseases. Anticarcinogenic efficacy of selenium compounds has been recently widely studied. The most active and useful selenium organic form can be obtained by humans from vegetal products. Selenium content in fruit and vegetables depends on its content in soil. Almost all soils of the world show a deficit in selenium, except the western regions of Canada, USA, Columbia, Venezuela, Australia, Israel and Ireland. In the Republic of Moldova, alike Romania, the soil is poor in selenium, and, subsequently, the necessary daily content of selenium might not be covered [121-123]. At the same time, it should be mentioned that the fertilizers containing selenium haven't a visible effect because of nitrates, chlorides and phosphates that bind it in insoluble compounds. In chernozems, selenium apears in accessible forms for plants.

The deficit of selenium in the human body, ascertained in some parts of the world, can be substituted through dietary diversification, fortification of food products during processing. Another method would be *biofortification*, the increase of bioavailable concentrations of essential elements in plant edible parts, by using fertilizers or physiologically active substances (*agronomic biofortification*) or by selecting plants with capacities of concentrating selenium (*genetic biofortification*). In this context, there were revealed a series of plants accumulating this oligo-element. The garlic (*Allium sativum* L.) has the capacity to accumulate the most necessary of such microelements, including selenium, but its content in garlic bulbs depends on its content in the soil and its accessibility for plants. Some researches proved that this element can be necessary for selenium accumulating plants and can contribute to yield increase.

The effect and action mechanisms of selenium haven't been fully descyphered yet, even if researches on this aspect have been lately intensified. Additionally, there have been undertaken studies on supplementing this essential oligoelement in plants and pharmaceutical preparations.

Within our research we have launched an experimental study on: a) the effect of gibberellins and of the coordination compound containing selenium, administered radicularly, on garlic plants biological performances; b) descyphering the mechanisms through which selenium as a microelement is involved in the antioxidating protection induced by oxygen reactive species. The objectives of experimental researches pursued: prospecting and validating some method for enriching *Allium sativum* L. plants with selenium, evaluating antioxidant protecting potentation of garlic plants after fortification with selenium. Literature shows data proving that selenium in small concentrations conditions the amplification of plant adaptive potential, diminishes drought negative impact, stabilizes the area of assimilating apparatus, reduces the drop of floral buds and contributes to the activization of growth processes during the repairing period, after improving humidity conditions [124, 125]. Selenium deficit in plants inhibits growth, retains flowering, reduces plant tolerance to environmental factors modification, generates chlorosis, turgor loss and withering.

Within IR spectrum of $[Co(DH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$ complex can be found the absorption bands of valence oscillations of $Co(DH)_2$ planar group deformation, cm^{-1}: $\nu_{as}(CH_3)$=2928, $\nu_s(CH_3)$=2871, $\nu_{as}(C{=}N)$=1546, $\delta_{as}(CH_3)$=1461, $\delta_s(CH_3)$=1376, $\nu_{as}(N{=}O)$=1237, $\nu_s(C{=}N)$=1285, $\nu_s(N{=}O)$=1083, $\gamma(OH)$=972, $\delta(CNO)$=730, $\nu_{as}(Co\text{-}N)$=507 and $\nu_s(Co\text{-}N)$=428. The complex belongs to cobalt (III) *trans*-dioximates class. The band characteristic of ($\delta(NH_2)$) is displaced in the region with higher frequencies (1631 cm^{-1}) in comparison with the band characteristic of free selenocarbamide [126]. The bands $\nu_{as}(BF_4)$=1084, $\nu_s(BF_4)$=761, $\delta(F–B–F)$=524 cm^{-1} belong to $[BF_4]^-$ anions of the external sphere [127].

The complex $[Co(DH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$ contains cations of $[Co(DmgH)_2(Seu)_2]^+$, $[Co(DmgH)_2(Seu)(Se\text{-}Seu)]^+$ and $[Co(DmgH)_2(Seu)(Se_2)]^+$ with a different completing coefficient and anions of $[BF_4]^-$ [128]. The octahedral coordination polyhedron of Co(III) is formed of four nitrogen atoms from two DH^- monodeprotonated anions, coordinated in a chelate mode with the formation of two metal cycles located in the equatorial plane. On the octahedron axial coordinates can be found two selenium atoms that belong to Seu, Se–Seu or Se–Se ligands (Fig. III.16). The intramolecular hydrogen bond formed between the two DH^- mono-anions is typical of the representatives of this class described in literature [129-132].

The results of the present study prove that, in the plants treated with both gibberellins and coordination compound $[Co(DH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$ – conventionally Fludisec, it was recorded the trend to improve the functional processes at the initial stages of ontogenesis and during vegetation period. The results are exposed in tables III.33–III.35.

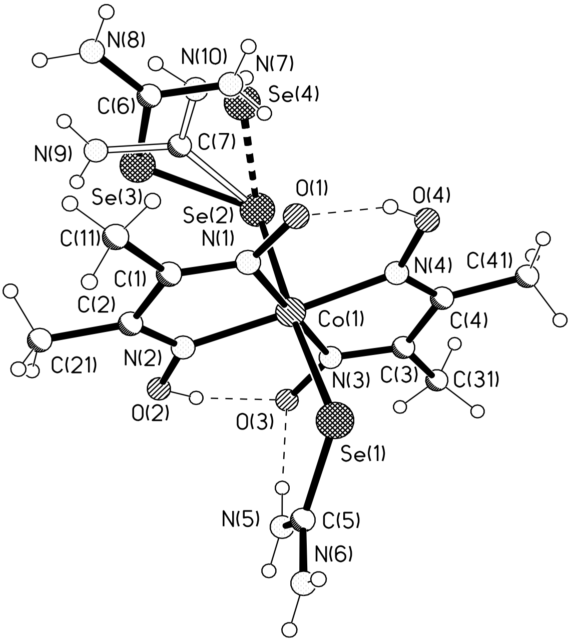

Fig. III.16. The structure of
$[Co(DH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se\text{-}Se)_{0.1}]^+$ complex. cation.

Table III.33. Morphological parameters of garlic plantlets from bulbs treated with PhAS.

Variants	Weight, mg			
	Coleoptile	Roots	Plantlet	Efficiency, % control
Control	0.63±0.017	0.25±0.005	0.88±0.017	100
Gibberellins, 125 mg·L^{-1}	0.66±0.013	0.26±0.006	0.92±0.015	104.54
Fludisec, 0.001%	0.57±0.011	0.23±0.003	0.80±0.020	90.91
Fludisec, 0.0001%	0.62±0.014	0.24±0.004	0.86±0.024	97.72
Fludisec, 0.00001%	**0.72±0.019**	**0.29±0.009**	**1.01±0.028**	**114.77**
Fludisec, 0.000001%	**0.77±0.022**	**0.32±0.007**	**1.09±0.031**	**123.86**
Fludisec, 0.0000001%	0.69±0.018	0.29±0.004	0.98±0.019	111.36

It has been established the property of the coordination compound with selenium to stimulate the growth processes of garlic plantlets at the initial stages of ontogenesis. A major effect was recorded within plantlets treated with Fludisec solution of 0.00001 and 0.000001%, respectively:

by 14–24% as compared to control, and by 9.8–18.5% as compared to the effect produced by gibberellins. It has been established that treated plantlets had a higher content of assimilating pigments, unlike control plantlets (Tab. III.34).

Table III.34. The content of assimilating pigments (mg/100 g subst.) in the leaves of garlic plants.

Variants	Weight, mg			
	Chlorophyll *a*	Chlorophyll *b*	Cl *a*+ Cl *b*	Carotenoids
Control	53.15±0.62	23.19±0.27	76.33±1.41	17.85±0.29
Gibberellins, 125 mg·L^{-1}	59.45±0.84	26.38±0.19	85.86±1.18	20.30±0.24
Fludisec, 0.001%	45.24±0.59	19.10±0,22	64.34±0.92	15.06±0.11
Fludisec, 0.0001%	65.44 ±0.67	**28.35**±0.30	93.80±0.77	19.95±0.18
Fludisec, 0.00001%	**66.81±0.98**	**28.58±0.48**	**95.39±1.23**	**22.57**±0.19
Fludisec, 0.000001%	**82.02±1.43**	**36.9±0.55**	**118.21±1.85**	**26.27**±0.32
Fludisec, 0.0000001%	56.81±1.04	27.31±0.43	84.12±1.02	16.78±0.20

The content of chlorophyll pigments in the leaves of plants treated with gibberellins exceeded that of control plantlets by 12.5%, while the content of assimilating pigments in the plantlets treated with Fludisec of 0.00001–0.000001% was by 25.0–5487 % higher.

In some recent studies [105, 134-135], it has been mentioned that Se is an antioxidant that activates protection mechanisms and reduces oxidative stress in plant chloroplasts, in stress conditions. Judging by the content of malonic dialdehyde, it has been established that both the gibberellins and the coordination compound with selenium reduce the effect of oxidative stress in plantlet leaves as a result of increasing the activity of antioxidant protection enzymes (Tab. III.35). The efficiency of antioxidant protection in the plantlets treated with Fludisec is higher than in the plantlets treated with Gibberellins, especially as compared to control plantlets. Investigation results show that bulb treatment with Fludisec stimulates growth processes, increases the content of assimilating pigments and intensifies antioxidant protection in garlic plantlets.

Table III.35. The content of malonic dialdehyde and the activity of antioxidant protection enzymes in garlic plantlets at initial stages of development.

Variants	MDA, mkM• g^{-1} f.w.		SOD, un. conv. • g^{-1} f.w.		CAT, mM• g^{-1} f.w.		APX, mM• g^{-1} f.w.	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	7.06± 0.22		7.34± 0.18		3.71± 0.15		3.62± 0.14	
Gibberellins, 125 mg·L^{-1}	6.29± 0.18	-10.91	10.60± 0.31	44.4	3.84± 0.12	3.5	5.40± 0.16	49.2
Fludisec, 0.0001%	4.83± 0.20	-31.59	13.93± 0.22	89.8	3.89± 0.16	4.8	5.18± 0.15	43.1
Fludisec, 0.00001%	4.66± 0.17	-33.99	14.04± 0.34	91.3	4.11± 0.14	10.8	5.47± 0.11	51.1
Fludisec, 0.000001%	4.66± 0.13	-33.99	14.50± 0.38	97.5	4.11± 0.11	10.8	6.29± 0.21	73.8
Fludisec, 0.0000001%	5.47± 0.21	-22.52	13.91± 0.11	89.5	3.17± 0.10	-14.5	4.07± 0.10	12.4

In field experiences it was studied the influence of Gibberellins vs that of Fludisec compound on antioxidant properties of garlic plant leaves and bulbs, on the content of selenium as a factor increasing antioxidant protection capacity, growth and plant productivity. The scheme of experiences includes the following variants: 1 – control; 2 – plants treated with Gibberllins solution 125 mg·L^{-1}, 3 – plants treated with Fludisec aqueous solution of 0.000001%. During vegetation, plant foliar apparatus has been treated 3 times with a 2 week interval. The analysis of physiological processes was achieved on leaves, after each treatment, and on bulbs, after the 2-nd and 3-rd treatments. After each treatment it has been established the content of assimilating pigments, intensity of oxidative stress (on the basis of malonic dialdehyde content), capacity of antioxidant protection systems, productivity and selenium content in leaves and bulbs. The results are exposed in tables III.36-III.40.

In many previous researches it had been proved that organs dehydration induced by environment adverse factors causes stomata closure, photosynthesis disorganization, inhibition of antioxidant protection mechanisms, intensification of oxygen reactive species production, fact affecting homeostasis and normal development of organism vital processes. Excessive formation of ROS conditions lipid peroxidation, protein oxidation, degradation of nucleic acids, enzyme inhibition, activation of programmed cell death [136]. When ROS concentration reaches threshhold level, lipid peroxidic oxidation (LPO) intensifies in all cell membranes, fact negatively influencing cell normal functioning. LPO worsens the state of oxidative stress by the fact that the radicals derived from LPO can interact with proteins and DNA [137, 138]. Unsaturated fatty acids are very sensitive to LPO attack because a single OH can peroxidate several polyunsaturated acids, being the reason of disruptive chain reactions of metabolic structures and processes. Taking into account the level of malonic dialdehyde (Tab. III.36), it can be infered that ROS production within the organs of garlic plants treated with Gibberllins, especially with Fludisec, is significantly more reduced as compared to control plants. The leaves and bulbs of control plants showed a higher content of MDA, about 32.35±0.38 şi 16.18±0.20 mkM·g.m.p. within the plants treated with gibberellins, MDA content reduced correspondingly by 13.08 and 7.18%. Under the influence of the coordination compound with selenium, MDA content reduced by 21.11% – in leaves, and by 25.15% – in bulbs (Tab. III.36). Investigation results proved that ROS had caused oxidative distructions, especially in control leaves and bulbs as compared to plants pretreated with Fludisec. Control plants show a lower capacity of antioxidant protection as compared to the plants supplied with Se.

In optimal conditions, within plant cells, it permanently takes place a certain level of peroxidic oxidation of lipids that maintains constantly due to the systems of antioxidant protection. An essential role in protecting cells against oxidative distruction devolves to enzymatic system, especially to superoxide dismutase, that catalizes superoxideradicals dismutase reaction ($O^{\cdot -}$). Interaction rate of SOD and $O^{\cdot -}$ depends on membrane viscosity, on the degree of cell hydration [140]. DSO activity changes in accordance with drought intensity – during a moderate drought, enzyme activity intensifies, during a long lasting drought, enzyme activity reduces [140, 141]. Experimental results reflected in table III.36, confirm the above mentioned facts.

The plants treated with Gibberllins and Fludisec showed an increase of SOD activity in leaves by 6.45 and 24.80% respectively; in bulbs – by 9.56 and – by 32.82% as compared to enzyme activity in the organs of control plants. The administration of the coordination compound with selenium ensured a better influence on the capacity of antioxidant protection that dispalyed through the intensification of antioxidant enzyme activity. The activity of SOD, CAT, APX, GPX and GR showed an increasing trend in both leaves and bulbs. The rapid intensification of DSO activity generates the increase of H_2O_2 content, fact leading to enzyme inhibition, that can produce much more damage, as hydrogen peroxide has the property to penetrate cell membranes and produce distant distructions from occurance place. SOD effective functioning is greatly determined by the function of other components of antioxidant protection system, particularly by those eliminating hydrogen peroxide (catalase, peroxidases), and by the enzymes of ascorbate cycle-glutation. Obtained data prove that the activity of both enzymes increases under the influence of gibberellins and potassium selenate.

Table III. 36. The content of malonic dialdehyde and the activity of antioxidant protection enzymes within garlic plant leaves and bulbs (*Allium sativum* L.).

Parameters	Control	Gibberellins, 125 mg·L^{-1}		Fludisec, 0,00001%	
	M ± m	M ± m	Δ, %	M ± m	Δ, %
In leaves (after the III-rd pre-treatment)					
MDA, mkM · g^{-1} f.w	32.35 ± 0.38	28.12 ± 0.29	-13.08	25.52±0.36	-21.11
SOD, un. conv.·g^{-1} f.w.	62.81 ± 0.73	66.86 ± 0.92	6.45	78.39±0.47	24.80
CAT, mmol · g^{-1} f.w.	1.30 ± 0.015	1.78 ± 0.009	36.92	1.94±0.04	49.23
APX, mmol · g^{-1} f.w.	8.43 ± 0.13	9.62 ± 0.17	14.12	12.44±0.35	47.57
GR, mM · g^{-1} f.w.	172.8 ± 2.08	195.31 ± 4.27	13.00	118.30±2.12	38.46
GPX, mM · g^{-1} f.w.	85.44 ± 1.94	106.58 ± 2.15	24.74	217.01±3.64	25.56
In bulbs (after the III-rd pre-treatment)					
MDA, mkM · g. m.p.	16.18 ± 0.20	15.18 ± 0.34	-7.18	12.1±0.19	-25.15
SOD, un. conv.·g^{-1} m.p.	52.81 ± 0.77	57.86 ± 0.61	9.56	70.14±0.52	32.82
CAT, mmol · g^{-1} m.p.	2.14 ± 0.012	2.32 ± 0.07	8.41	2.98±0.05	39.25
APX, mmol · g^{-1} m.p.	7.16 ±0.10	8.57 ± 0.14	19.69	11.31±0.02	57.96
GR, mM · g^{-1} m.p.	182.84 ±2.95	205.31 ± 5.62	12.29	227.01±3.89	24.16
GPX, mM · g^{-1} m.p.	95.15 ± 2.31	101.34 ± 1.87	6.51	120.21±2.05	26.34

It’s worth mentioning that in plants pretreated with gibberellins, it was revealed a stronger CAT activization, while in those treated with selenium and Fludisec, it was noted a significant increase of catalase and ascorbateperoxidase. Similar results were obtained at determining the modification degree of GPX and GR activity, fact indicating on enzyme different roles in reducing hydrogen peroxide and in maintaining H_2O_2 level.

The character of changes affecting the content of malonic dialdehyde and the activity of antioxidant protection enzymes in treated plants confirm the effect of oxidative stress diminuation indused by selenium. Selenium antioxidant action displays through the tendency of normalizing these parameters; MDA values significantly decreased as compared to both untreated plants, to gibberllins effect.

The consequences of oxidative stress, caused by drought are: the abundant generation of singlet oxygen, chloroplast distruction and the decrease of assimilating pigments in leaves. According to the data obtained by us, the provision of garlic plants with selenium, by treating foliar apparatus with Fludisec, generates the increase of assimilating pigments content (Tab. III.37). The plants treated with solution of gibberllins showed a content of green pigments summarily by 17.61% higher than control plants, while in the plants treated with Fludisec, the content of green pigments was by 24.81% higher.

Table III.37. The influence of PhAS on the content of pigments in the leaves of garlic plants.

Variants	Chlorophyll *a*, mg · 100 g m. p.		Chlorophyll *b*, mg · 100 g m. p.		Chlorophyll *a+b*, mg · 100 g m. p.		Chlorophyll, mg · 100 g m. p.	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	20.21±0.51		9.49±0.16		29.70±0.66		8.76±0.20	
Gibberellins, 125 mg·L^{-1}	23.81±0.62	17.81	11.11±0.28	17.07	34.93±0.81	17.61	10.13±0.22	15.64
Fludisec, 0.00001%	25.17±0.54	24.54	11.78±0.29	24.13	37.07±0.66	24.81	10.30±0.17	17.58

It comes into notice the fact the plants treated with Fludisec displayed an essential increase in carotenoids content, whose function is to protect chloroplasts from oxidative distruction. The effect of potassium selenate on the content of assimilating pigments can be explained by the fact that Se participates in the reactions of chlorophyll synthesis, tricarboxylic acids synthesis and in the metabolism of fat acids with a high molecular weight [142, 143]. The protecting action of selenium compound displays through the regulation of proline content, which, as we know, has multiple beneficial functions as related to plant response to stress factors, including osmotic adjustment, stabilization of cell and membrane structure and elimination of free radicals. So far, it isn't clear through which mechanisms the increase of proline content is directly connected to selenium action. It can be assumed that Pro increase depends on carbohydrates accumulation, as a result of optimizing the content of assimilating pigments and photosynthesis, as it is known the fact that such carbohydrates like glucose, fructose, mannitol, and, in particular, saccharose, play a significant role in the accumulation of proline [144]. We consider that proline accumulation in conditions of stress can be the result of its *de novo* synthesis or of its low degradation, either the result of affecting its transportation from an organ to another, or the result of all these changes.

It has been found a major influence of Fludisec compound on proline content in the leaves and bulbs of *Allium sativum* L. plants (Tab. III.38). Foliar pretreatment of plants with Fludisec solution conditioned a higher increase of proline content in organs as compared to the effect of gibberellins.

Table III.38. Proline content (µg · g^{-1} m.p.) in the leaves and bulbs of *Allium sativum* L. plants.

Organ	Control	Gibberellins, 125 mg·L^{-1}		Fludisec, 0.00001%	
	M±m	M±m	Δ, % control	M±m	Δ, % control
In leaves	0.240 ± 0.006	0.293 ± 0.008	**22.08**	0.410 ±0.013	**70.83**
In bulbs	1.735 ± 0.047	1.850 ± 0.038	**6.63**	2.247 ±0.052	**29.51**

It is considered that the intensification of antioxidant properties is due to the increase of selenium content in plant organs, which, in their turn are in positive linear correlation with selenium content in the soil. Selenium absorption by plants depends on several factors, but when Se is present in a soluble form, it's quickly absorbed by plants, despite the fact that the differences between plant species are very obvious. In this sense, the plants were divided into three categories: 1 – accumulating plants (from 0.05 to 0.25 mg/kg); 2 – plants absorbing average amounts, up 100 pg Se/kg, and 3 – plants that are not accumulating, containing less than 30 pg Se/kg, in field conditions [133]. The authors also note that the safety margin of Se average concentrations in the plant is quite narrow. Most plants have a low content of Se, less than 25 µg/kg.

Some mono- and dicotyledonous plant species have the capacity to absorb Se through leaf surface, being then transported and accumulated in roots in various forms – inorganic selenite and organic compounds. This postulate was confirmed in our investigations. Foliar treatment of garlic plant with gibberellins, which enhances Se absorption from soil [145], and with potassium selenate has resulted in the increase of Se content in the leaves and bulbs of plants (Tab. III.39). The major effect of selenium increase in both leaves and bulbs was recorded in plants treated with Fludisec solution.

Table III.39. The effect of garlic plants treatment on selenium content in leaves and bulbs ($\mu g.kg^{-1}$ s. u.).

Organ	Control	Gibberellins, 125 mg·L^{-1}		Fludisec, 0.00001%	
	M±m	M±m	Δ, % control	M±m	Δ, % control
leaves	74.0 ± 1.8	84.0 ± 2.1	13.51	88.0 ± 1.9	18.92
bulbs	47.0 ± 0.7	59.0 ± 1.2	25.53	70.0 ± 1.1	48.94

The content of selenium in leaves and bulbs of plants treated with gibberellins showed an increase of 10.0 and 12.0 µg·kg of f.w. as compared to selenium content in the organs of control plants. Considering the content of Se, especially in bulbs, the plants supplemented with Fludisec prevail to plants treated with gibberellins.

Some authors are of the opinion that selenium, participating in chloroplast formation and chlorophyll synthesis, influences the intensity of photosynthesis, thus ensuring a better productivity [143, 146]. Recent studies with some cereal and vegetable crops prove that Se supplement stimulates their growth rate [133]. Optimizing plant functional status throughout foliar pre-treatment with gibberellins, and especially with Fludisec, resulted in the stimulation of plant growth processes and biomass accumulation (Tab. III.40).

Table III.40. The effect of Gibberellins and Fludisec on the productivity of garlic plants.

Variants	Plant weight $g \cdot pl^{-1}$		Plant productivity, g/pl.		Yield, kg/m^2	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	64.44 ± 0.82		36.289 ± 0.73		1.016 ± 0.09	
Gibberellins, 125 $mg{\cdot}L^{-1}$	68.87 ± 1.08	6.87	39.000 ± 0.58	7.47	1.092 ± 0.13	7.48
Fludisec, 0.00001%	78.10 ± 0.74	21.20	44.34 ± 0.64	22.20	1.241 ± 0.08	22.18

Biomass accumulation of plants pre-treated with gibberellins, especially with Fludisec, is much higher than that of control plants. The effect of increasing plant weight is 6.87 and 21.20% respectively. The productivity of plants treated with Gibberellins and Fludisec override control plants by 7.47 and 22.20% respectively. Plant treatment with a coordinate compound with selenium ensured a production increase by 22.18% as compared to control plants and by 13.70% as compared to to the yield obtained from plants treated with gibberellins.

Thus, the coordination compound of $[Co(DH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se_2)_{0.1}][BF_4]$ has properties of a physiologically active substance, which display through the stimulation of growth processes, increase of antioxidant protection capacity of plant organs by intensifying the activity of antioxidant enzymes with a positive impact on plant productivity.

Therefore, we can conclude that selenium deficiency in the soil, that leads to low plant productivity and selenium deficit in animals and humans, can be substituted by treating plant leaf area with physiologically active substances or coordination compounds containing selenium. At pre-treatment, garlic plants (*Allium sativum* L.) actively accumulate selenium, fact connected to the presence in cells of glycosides containing sulfur that can be replaced by a Se atom with similar properties. Plants supplementation with selenium results in optimizing the achievement of plant adaptive potential, which manifests through the intensification of antioxidant properties, increase of photosynthetic pigments fund, accumulation of biomass and plant productivity. The leaves and bulbs of garlic plants supplemented with selenium are characterized by increased antioxidant properties.

Conclusions:

1. Coordination compounds with oxide ligands possess features of bioactive substances with a positive impact on plant growth, development, resistence and productivity.
2. The treatment of sowing seeds and foliar apparatus during vegetation with aqueous solutions of Difecoden, Difemanden, Coditiaz, Conimid and Cobamid conditions the optimization of functional status, growth and development of corn, soy, sugar beet, cucumber, tomato, beet, peanut plants both in favorable conditions of humidity and in conditions of a moderate water deficit.
3. The coordination compounds under use have the property to activate vital processes at the initial stages of plant individual development, stimulate the radicular system and the sprout growth, ensures water satus homeostating in conditions of suboptimal hydric regime, increases plant productivity, consolidate the organism protective functions, increasing tolerance to suboptimal factors.
4. In conditions of reduced humidity, Difecoden, Cobamid, Conimid and Coditiaz diminish drought effect on assimilating apparatus formation, biomass accumulation and plant yield. The use of PhAS containing in

their molecule nutritive elements, ensures the stabilization of plant production process.

5. The use of coordination compounds in combination with hydrosoluble polymers (PVP, coVP) ensures the reduction of water use during transpiration, without negative consequences on plant biomass accumulation.
6. Difecoden, Conimid and Fludisec coordination compounds possess antioxidant properties which manifest through the increase of antioxidant protection capacity of the organs of treated plants, as a consequence of intensifying the activity of antioxidant enzymes that have a positive impact on plant productivity.

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Chapter IV.

PERSPECTIVES OF USING COORDINATION COMPOUNDS BASED ON DIOXIME LIGANDS IN IDUSTRIAL PROCESSES

IV.1. Coordination compounds as dyes in the textile industry

One of the important tasks of contemporary chemical synthesis is to obtain new materials with useful properties and to determine the various areas of their use.

Coordination compounds are used in the processes of industrial dyeing of cotton fabrics [1]. With the aim to increase the intensity of fabric dyeing and improve the quality of this process, there have been synthesized cobalt(III) dioximates with thio- and selenocarbamide, which have been tested as catalysts for the process of reducing dyes. The use of the proceeding based on these complexes contributed to the increase of material staining intensity from 80.1-90.1 up to 94.1-98% [2].

It is known the process of dyeing textile materials on the basis of cobalt(III) dimethylglyoximates with amine or sodium formaldehyde sulfoxylate (rongalite C) [1]. The drawback of the process is dyeing low intensity (83-88.7%) [1]. The process has been optimized by changing the quantities of cobalt salts and dimethylglyoxime, that increased dyeing intensity from 90.8 to 97.8% [3].

Another drawback of known dyes is the duration of their fixation on the fabric as a result of insufficient activity of used catalysts. The use of catalysts in dyes reduces their effect as a result of rongalite C low stability in their presence, which leads to additional waste of dye and loss of fabric staining intensity. It has been developed a more efficient process by using mixtures containing cobalt(III) dimethylglyoximates, that leads to the increase of dye fixation degree on the material by 16-20%, stability of dye solution from 1.5 to 24 hours and acceleration of dye fixation process from 2-3 min to 30-60 sec [4]. In addition, the proposed mixture, unlike the ordinary one, allows to reduce twice the quantity of used rongalite C and sodium hydroxide.

Deciphering the crystal structure of $[Co(DH)_2(Thio)_2]NO_3 \cdot H_2O$ complex allowed the elucidation of some molecular structure peculiarities and the elaboration of complex application methods in industrial processes of dyeing textile materials [5].

A series of complexes based on various dioximes have been tested as catalysts for reduction reactions of dyestuffs [6].

IV.2. Coordination compounds as inhibitors of steel corrosion processes in water

The study concerns metal protection against corrosion in water. Its results can be applied for inhibiting steel corrosion in different closed systems, such as in water pipes. Natural water or that used for technological purposes, comprising activating ions, chloride, sulfate presents a relatively aggressive environment, where steel corrosion occurs at a high speed. Thus, in the water from Chişinău water pipes, containing mg/L: Ca^{2+} – 42.5, Mg^{2+} – 19.5, HCO_3^- – 97.6, SO_4^{2-} – 203.7, Cl^- – 56.7 with a total amount of salts of 0.457 g/L, the speed of steel corrosion, within 8 hours, is rather high, and reaches about 21 $g/m^2 \cdot day$. At increasing exposure time, corrosion speed decreases due to the formation of corrosion products and calcite $CaCO_3$ on the etched surface of the oxidation-hydroxide film.

The ions of SO_4^{2-} cause a rather uniform general corrosion, while on the internal surface of pipes can form pitting on the account of chloride activating ions present in water that, in a series of cases, can perforate the pipes and produce damage. More than that, ionized iron, passing in water, accumulates and diminishes its quality.

One of the ways to inhibit corrosion processes is the use of chemical compounds with the aim to increase steel resistance to corrosion.

The complex of $[Zn_2(CH_3COO)_4(NioxH_2)_2(bpy)(H_2O)_2]$ ($NioxH_2$ – 1,2-cyclohexanedione dioxime; bpy – bipyridyl) was tested as inhibitor of steel corrosion in closed systems of water pipes. The testing, in corrosion conditions, of samples with the dimensions of 50x25x3 mm is carried out by their complete immersion in solution, at the same depth, with air access. Roughness is created by grinding samples. The losses caused by corrosion are recorded gravimetrically. The effect of inhibitor action is qualitatively evaluated according to speed k, $g/m^2 \cdot day$ and value of braking coefficient $\gamma = k/k_1$, where k_1, k – the speed of metal corrosion with and without inhibitor. This coefficient shows how many times corrosion speed decreases as a result of inhibitor action. The influence of inhibitor concentration and testing time on corrosion speed k, $g/m^2 \cdot day$ and braking coefficient γ are exposed in table IV.1. The data show that the greatest effect is obtained while using the inhibitor in the concentration of 0.05-1.0 g/L. Thus, at inhibitor concentration of 0.25 g/L, and experiment duration of 72 hours, the losses caused by corrosion decrease 7.1 times. At inhibitor concentration of 0.5 g/L and the same experiment duration, the losses caused by corrosion decrease 9.4 times.

The quantity of inhibitor introduced in the corrosive medium has a determining role. The lowest limit is the concentration of 0.05 g/L, because at a smaller amount of inhibitor in the corrosive medium, the decrease of losses is not significant. The highest limit of inhibitor concentration is that of 1.0 g/L for, along with subsequent concentration increase, corrosive losses slightly modify, while the economical losses increase on the account of the large amount of used inhibitor.

Table IV.1.

The influence of inhibitor supplement on the parameters of steel Ст. 3 corrosive process in water.

Inhibitor concentration, g/L	Experiment duration (τ), hours	Corrosion speed (k), $g/m^2 \cdot day$	Braking coefficent (γ)
0.0	8 24 72 240	21.0 12.0 6.6 4.0	- - - -
0.05	8 24 72 240	6.35 2,65 1.73 1.15	3.3 4.5 3.8 3.5
0.1	8 24 72 240	6.28 2.22 1.66 1.07	3.3 5.4 4.0 3.8
0.25	8 24 72 240	4.25 2.55 0.93 0.57	4.9 4.7 7.1 7.1
0.5	8 24 72 240	5.48 2.52 0.7 0.58	3.8 4.8 **9.4** 6.9
0.75	8 24 72 240	5.65 2.64 0.73 0.62	3.7 4.6 8.5 6.5
1.0	8 24 72 240	5.75 2.73 0.82 0.72	3.7 4.4 8.1 5.6

Thus, it was developed a new, efficient, economical and ecological inhibitor of steel corrosion in water, that can significantly reduce the losses caused by corrosion processes [7].

IV.3. Dioximates of iron(II) and iron(III) as catalysts and stabilizers in polyurethane compositions

Polyurethane compositions are widely used. They can be used to tick metal parts, to obtain decorative materials, lakes with different destination etc.

The ordinary polyurethane compositions contain substances fulfilling the role of catalysts (for example, tin octoate) and substances playing the role of stabilizers (for example, butyl-p-cresol). Iron dioximates with pyridine and some of its derivatives fulfil simultaneously the functions of catalyst and stabilizer. Polyurethane composition includes:

polyether with hydroxyl groups	70-80 parts by weight (pbw)
diisocyanate	18 – 34
white soot	10 – 38
iron dioximate	0.11 – 0.9
castor oil	8 – 12.

This composition was prepared according to the described method [8]. 75 parts by weight of poly diethylene glycol adipate, dried to the humidity of 0.04-0.07%, 10 parts by weight of castor oil, and 0.03 parts by weight of catalyst-stabilizer – iron dioximate are introduced in a Bekken type stirrer. The obtained mixture is stirred at 80-90° C to the total dissolution of iron dioximate. Further, 25 parts by weight of white soot filling are added in the stirrer cooled to 20-25° C. The mixture is stirred in vacuum during 20-40 minutes, then 22 parts by weight of 4,4′-diphenylmethanediisocyanate are added and the obtained mixture is stirred for other 12-25 minutes in vacuum. This composition is used to manufacture polyurethane adhesives metallic parts that apply to construction machinery industry. It is also used to produce white decorative material with plane surface.

To stick axle boxes, it was used another polyurethane composition that distinguishes from the previous one through the weight ratio of ingredients. In that case, 70 parts by weight of poly diethylene glycol adipate, dried to the humidity of 0.04-0.07%, 8 parts by weight of castor oil, and 0.01 parts by weight of catalyst-stabilizer are introduced in the

Bekken stirrer and stirred at the temperature of 80-90° C to the total dissolution of the catalyst. Then, 10 parts by weight of diphenylmethane-4 4'-diisocyanate are added in the cooled mixture and the latter is stirred for 12-15 minutes in vacuum.

The results of testing polyurethane compositions, obtained on the basis of iron dioximates, are exposed in Table IV.2 [9].

Table IV.2.

The properties of polyurethane compositions based on iron dioximates.

№ d/o	Catalyst	Viability at 20° C	Solidification time	Adhesiveness to steel, kg s/cm^2		
				Till ageing	After ageing	Mineral fraction content, %
1	Without catalyst	2.0	1.0	10.0	9.2	16.0
2	Tin octoate [8]	0.2	4.0	9.9	7.3	12.5
3	$[Fe^{II}(DfH)_2(Py)_2]$	3.0	3.0	54.0	52.0	6.2
4	$[Fe^{II}(DfH)_2(3\text{-}CH_3\text{-}Py)_2]$	2.5	2.0	53.0	51.0	5.4
5	$[Fe^{II}(DfH)_2(4\text{-}CH_3\text{-}Py)_2]$	2.6	2.5	52.0	52.0	5.2
6	$[Fe^{II}(DfH)_2(3\text{-}CONH_2\text{-}Py)_2]$	2.7	3.5	51.5	50.5	4.8
7	$[Fe^{II}(DH)_2(Py)_2]$	2.9	3.5	52.6	51.8	4.9
8	$[Fe^{III}(DH)_2(Py)_2]I_3$	3.7	3.5	54.6	55.0	4.5
9	$[Fe^{III}(DfH)_2(Py)_2]Cl\cdot 1.5Cl_2$	4.2	4.0	53.4	53.2	5.6
10	$[Fe^{III}(DfH)_2(Py)_2]Br_3$	5.0	4.0	50.0	50.0	6.1
11	$[Fe^{III}(DfH)_2(Py)_2]I_5$	4.0	3.5	52.3	53.0	4.6

Epoxy compositions created on the basis of iron(II) and (III) dioximates are characterized by a higher viability, compared to those created on the basis of tin octoate. They also contribute to the increase of compositions adhesiveness to steel. Iron dioximates confer to epoxy compositions high stability to thermooxidative destruction.

It's important the fact that the iron dioximates under study play a double role in epoxy compositions: that of oxidizer and stabilizer, property that radically distinguishes them from known catalysts and stabilizers used in such compositions.

IV.4. Complex combinations as dielectric material

Judging by the structure of complex combinations of researched metals, it has been assumed that these compounds manifest dielectric properties. In order to test this hypothesis, it was measured the specific resistance of the following single crystals $[Co(NioxH_2)_2Cl_2]$, $[Ni(NioxH_2)_2Cl_2]$, $[Cu(NioxH_2)_2Cl_2]$ [10], $[Cu^I(NioxH_2)_2I]_n$ [11], $[Fe(Dena)_4Cl_2(DH_2)]_n$ and of $[Fe(DfH)_2(DMSO)_2]\cdot H_2O$ tablet [13] (the tablet represents the pressed polycrystalline compound).

The resistance of researched samples was measured at room temperature, with the help of direct current bridging of P 4053 type. The voltage was applied to two parallel surfaces with equal sides of prismatic crystals. Linear dimensions of single crystals were measured using the micrometer of MKB type. Calculations of specific resistance were made according to $\rho=RS/l$ formula, where R is sample resistance, S is the area of faces to which voltage was applied, and l is the crystal length. The experimental values of coordination compounds specific resistance are exposed in Table IV.3. The data show that the specific resistance of researched compounds is higher than that of glass, china and synthetic sapphire, being commensurate with specific resistance of fused quartz and mica, widely used as dielectric materials.

Du to the marked properties of dielectric material, these compounds can be applied in electrotechnics.

Table IV.3.

Specific resistance values of Fe, Co, Ni and Cu compounds
with α-dioximes.

Compound	R, Ohm	S, cm^2	*l*, cm	ρ, Ohm/cm
$[CoCl_2(NioxH_2)_2]$	$2\cdot10^{12}$	$1.05\cdot10^{-2}$	$5\cdot10^{-2}$	$4.2\cdot10^{11}$
$[NiCl_2(NioxH_2)_2]$	$2.7\cdot10^{12}$	$3.6\cdot10^{-3}$	$6\cdot10^{-2}$	$1.6\cdot10^{11}$
$[Cu^{II}Cl_2(NioxH_2)_2]$	$1.7\cdot10^{13}$	$1.4\cdot10^{-2}$	$2\cdot10^{-2}$	$1.2\cdot10^{13}$
$[Cu^{I}I(NioxH_2)]_n$	$4.0\cdot10^{10}$	$2.5\cdot10^{-3}$	$5\cdot10^{-2}$	$\sim5\cdot10^{10}$
$[Fe(DfH)_2(DMSO)_2\cdot H_2O$				$4\cdot10^{10}$
Glass				10^7
Porcelain				10^8
Synthetic sapphire				10^{10}
Fused quartz				10^{11}
Mica				10^{13}

IV.5. Microbial innovational technologies for producing hydrolytic enzymes

Micromycetes of *Aspergillus, Penicillium, Rhizopus, Trichoderma* and *Fusarium* genera cover the most perspective producers of exocellular hydrolases, able to achieve the synthesis of enzymes within a short development cycle on cheap and affordable media [14-27].

The analysis and systematization of research, achieved within previous years (2001-2014), concerning the biosynthetic capacity increase of fungi strains producing exocellular hydrolytic enzymes revealed the coordinatively bound 3*d* elements (Zn, Co, Ni, Cu, V, Fe etc.) as remarkable stimulators and regulators of enzyme genesis processes in micromycetes [28-47].

Hydrolases (amylase, pectinase, protease, lipase and cellulase) are one of the most important groups of enzymes, widely applied in various industrial processes, agriculture, environment protection, ecological and medical diagnosis, pharmaceutics, scientific investigation, human and veterinary medicine etc. Given the recent trend of developing environmentally friendly technologies, enzymes are extensively used in the processes of environment bioremediation, biodegradable detergents production, substitution of aggressive chemical reagents within technological processes of raw material processing (use of renewable energy sources and biofuels, leather processing) [48-59].

The biotechnologic attainment of enzymatic preparations supposes the maximum exploitation of microorganisms biosynthetic potential. Previous research on increasing the biosynthetic capacity of fungi strains that produce exocellular hydrolases had highlighted, for each producer strain, the complex combinations of metals that, except the enzymatic activity increasing effect, accelerate the manifestation of enzyme biosynthesis maximum, differentiatedly influences the synthesis of components within the enzymatic complex synthesized by the producer strain, fact offering opportunities to obtain final products with a forecast composition. As stimulators of hydrolase activity, a special interest represent the coordination compounds of cobalt, copper, zinc and iron based on different oxime ligands.

Thus, the introduction of $[Co(MH)_2(Py)_2][BF_4]\cdot H_2O$ metal complex in the cultvation medium of *Aspergillus niger* CNMN FD 01 strain – lipase producer – increased the strain enzymatic activity by 30.0-46.2%, while $[Co(DH)_2(An)_2][BF_4]$ and $[Co(NioxH)_2(An)_2][BF_4]$ coordination compounds enhanced the process of lipase synthesis by 88% and 41-112% respectively [42, 60]. For the strains of *Rhizopus arrhizus* Fisher C – pectinase producer, and *Aspergillus niger 33* CNMN FD 06A – amylase producer, cobalt(III) compounds $[Co(DH)_2(Thio)_2]_3F[SiF_6]\cdot 1.5H_2O$, $[Co(DH)_2(Thio)_2]_2[SiF_6]\cdot 3H_2O$, $[Co(DH)_2(Thio)_2][BF_4]\cdot 3H_2O$ manifested as efficient stimulators that increased the enzymatic activity by 97.1-115.3% and 26.3-42.6% respectively, in under applied concentration and strain origin [61]. The introduction of $[Co(DH)_2(An)_2$

$]_2[TiF_6]\cdot 2H_2O$, $[Co(NioxH)_2(Sam)_2]_2[TiF_6]\cdot 3H_2O$ and $[Co(NioxH)_2(An)_2]_2[TiF_6]\cdot 3H_2O$ coordination compounds in the culture medium of *Aspergillus niger* 33-19 CNMN FD 02A strain, in the concentration of 5-10 mg/L, increases amylase biosynthesis by 23-64% and reduces the biosynthesis cycle by 24-48 hours [62, 63].

The coordination compounds of cobalt $[Co(DH)_2(Seu)_{7/4}(Se\text{-}Seu)_{1/4}]_2[TiF_6]\cdot H_2O$ (**1**), copper with disulfanilamide glyoxime $[Cu(DSamH_2)_3]SO_4$ (**2**) and zinc with 4,4-bipyridyl $[Zn_2(CH_3COO)_4(NioxH_2)_2bpy(H_2O)_2]$ exerted a biostimulating effect on protease biosynthesis in the strains of *Trichoderma koningii* Oudemans CNMN FD 15 and *Fusarium gibbosum* CNMN FD 12 micromycetes. While adding the complex of Cu(II) in the micromycete nutrient medium, in the concentration of 5-10 mg/L, the activity of neutral protease becomes higher in comparison with control during the whole cultivation period – on days 8-10 – fact characterizing the complex as a specific stimulator of neutral protease in *Trichoderma koningii* Oudemans. The early emergence of neutral protease in higher amounts indicates on biosynthesis increase mechanism throughout intensification of culture development and multiplication, due to the presence of metal complex in the culture medium.

The bionuclear complex of Zn(II) shows an obvious biostimulating influence on acid and neutral proteases and accelerates the manifestation of biosynthesis maximum, fact indicating on the intensification action of culture development and multiplication exerted by the metal complex. On the 8-th cultivation day, in all tested concentrations, the activity of both acid and neutral proteases becomes superior to the activity of control variant in the same day and to the maximum biosynthesis for control in general.

The coordination compound $[Zn_2(NioxH_2)_2(CH_3COO)_4bpy(H_2O)_2]$ included in the culture medium of *Fusarium gibbosum* CNMN FD 12 strain, displays a differentiated influence on the components of the proteolytic complex, synthesized by the micromycete: at the concentration of 5.0 mg/L, it determines the increase of acid protease activity, while the concentration of 15.0 mg/L increases neutral protease activity [38-40, 60-66].

Basing on obtained results, there have been developed a series of innovational proceedings and technologies for attaining hydrolytic enzymatic preparations of fungal origin through oriented synthesis. The integrated description and schemes for performing certain developed proceedings and technologies are exposed below.

Proceedings of hydrolytic enzyme oriented synthesis at submerse cultivation of producer strains in presence of chemical regulators.

A. Proceedings of amylolytic enzyme oriented synthesis at submerse cultivation of *Aspergillus* genus producer strains in presence of chemical regulators.

A.1. *The proceeding of amylase oriented synthesis with Aspergillus niger 57 (Aspergillus sp.) producer strain* [67].

As a prospective regulator of amylolytic activity for *Aspergillus niger* 57 micromycete, it was selected the cobalt dioximate with a hexafluorotitanate anion in the external sphere – $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ that, in the concentration of 15 mg/L, ensures the technologic cycle reduction by 24 hours, keeping the maximum level of enzymatic biosynthesis at the level of control variant (Table IV.4).

Table IV.4. The influence of $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ compound on the amylolytic activity of *Aspergillus niger* 57 micromycete.

Variants	Activity of ordinary amylases, M±m, u/mL		Activity of acid stable amylases, M±m, u/mL	
	On day 6	On day 7	On day 6	On day 7
$[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ conc. 15 mg/L	94.95±0.98	64.82±1.97	136.96±1.66	115.72±2.51
Control	45.87±2.51	95.58±1.97	86.57±2.50	134.12±1.45

Proceeding description. The proceeding includes the submerse cultivation of *Aspergillus niger* 57 micromycete in the presence of $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ coordination compound.

The basic nutrient medium for cultivating the fungal strain of *Aspergillus niger* 57, after sterilization (Concurrently with culture inoculation), is supplemented with $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ compound in the following ratio of ingredients, g/L: starch – 30.0 or cornflour – 50.0; yeast extract – 10.0; $NaNO_3$ – 9.0; $FeSO_4$ – 0.01; KH_2PO_4 – 1.0; $MgSO_4\cdot 7H_2O$ – 0.5; KCl – 0.5; $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ – 0.015, water – 1.0 L, pH – 4.7.

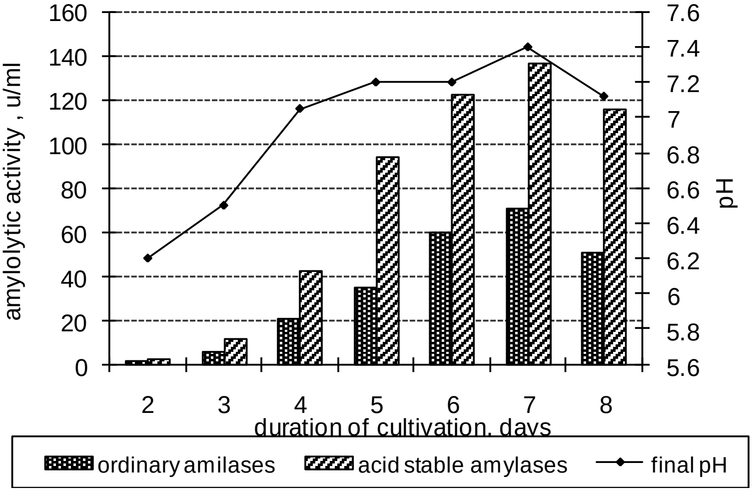


Fig. IV.1. Modification in strain amylolytic activity and biosynthesis medium pH in *Aspergillus niger* 57 strain in classical cultivation.

Micromycete cultivation is carried out in Erlenmayer flasks of 0.5 L capacity, containing 0.1 L liquid medium, on rotary shakers (180 r.p.m.), at a temperature of $28\text{-}30^0$ C. The cultivation duration is 6 days, while cultivation duration in classical conditions (control variant) lasts 7 days.

The proceeding advantage consists in the intensification of micromycete full development cycle and the acceleration of biosynthesis maximum manifestation: on the 6-th cultivation day – in the experimental variant, and on the 7-th cultivation day – in the control variant, for both types of amylase, while maintaining the maximum level of amylolytic activity. The proceedings permit to obtain an amylolytic enzyme preparation with high technologic characteristics and can be recommended for cleaving starch and starch components in different branches of economy – confectionery, bakery, brewery, production of alcoholic beverages and medicine.

A.2. *The proceeding of amylase oriented synthesis by Aspergillus niger CNMN FD 06 producer strain* [61, 67].

The basic nutrient medium for cultivating the fungal strains of *Aspergillus niger* CNMN FD 06 with the composition (g/L): starch – 30; bean flour – 9.0; wheat bran – 18.0; $MgSO_4$ – 0.5; KH_2PO_4 – 2.0; KCl – 0.5; water – 1.0 L; pH – 5.0, after sterilization, concurrently with the seed material, is supplemented with $[Co(DH)_2(Thio)_2][BF_4]\cdot 3H_2O$ coordination compound, in the concentration of 0.005…0.040 g/L. Cultivation is achieved in conditions of continuous shaking (180-200 r.p.m.), at a temperature of 28-30° C, cultivation duration – 4-5 days. Producer cultivation, according to the described proceeding, ensures the growth of amylolytic activity in the strain of *Aspergillus niger* 33-19 CNMN FD 06 by 23.7-28.9%, as compared to control variant on the day of maximum biosynthesis, the technologic cycle being reduced with 24-48 hours.

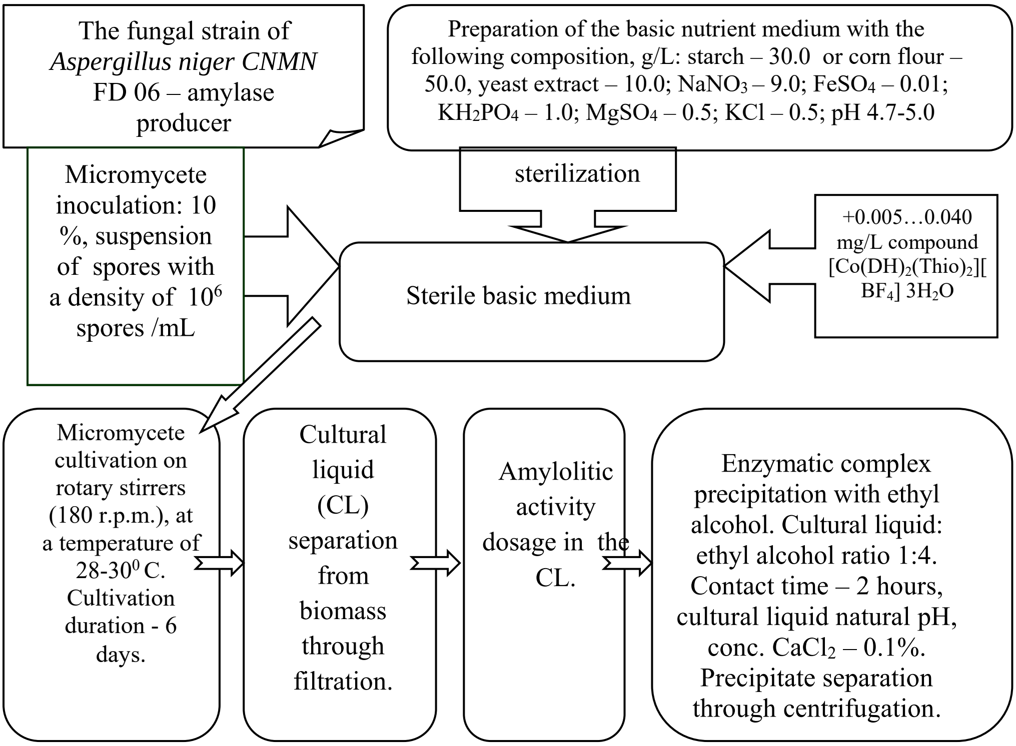
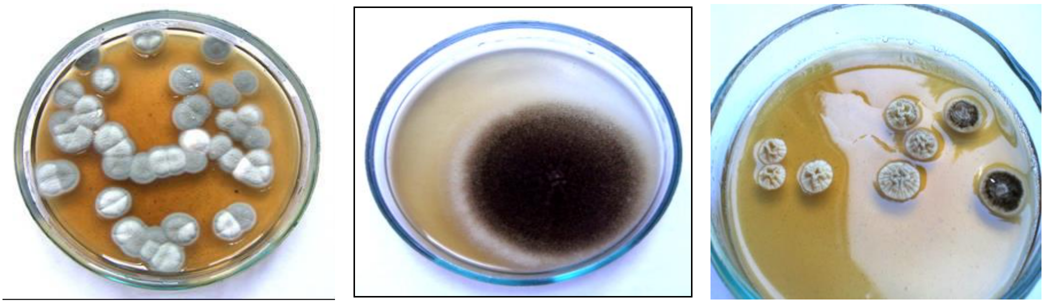


Fig. IV.2. The scheme for performing amylase oriented synthesis by *Aspergillus niger* CNMN FD 06 micromycete with chemical regulator application.



a b c

Fig. IV.3. The morpho-cultural aspect of *Aspergillus* strains – amylase producers, on the malt agar medium: a) *Aspergillus niger* 57; b) *Aspergillus niger* CNMN FD 02; c) *Aspergillus niger* CNMN FD 06 [61, 66, 68].

A.3. *Proceedings of amylase oriented synthesis by Aspergillus niger 33-19 CNMN FD 02 producer strain* [69].

For *Aspergillus niger* 33-19 CNMN FD 02A strain, within preliminary research, there were selected as efficient stimulators the coordination compounds containing Co(III) dioximates with the complex fluorinated anion of $[TiF_6]^{2-}$: $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$; $[Co(NioxH)_2(An)_2]_2[TiF_6]\cdot 4H_2O$; and $[Co(NioxH)_2(Sam)_2]_2[TiF_6]\cdot 3H_2O$, that can be used as an alternative. The compounds differ as regards the internal sphere composition.

Basing on the selected complexes, there have been elaborated three proceedings of producer strain submerse cultivation, further used in developing the improved technology of amylase attainment through oriented microbial synthesis.

***Proceeding I*: The basic nutrient medium for cultivating *Aspergillus niger* 33-19 CNMN FD 02A fungal strains in submerse culture, after sterilization, is supplemented with $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ coordination compound in the concentration of 5.0…10.0 mg/L, simultaneously with the inoculum material. Cultivation is performed by shacing (180-200 r.p.m.), flask tehnology at a temperature of 28-30° C, cultivation duration – 4-5 days. Nutrient medium composition (g/L): starch – 3.0; bean flour – 9.0; wheat bran – 18.0; $[Co(DH)_2(An)_2]_2[TiF_6]\cdot 2H_2O$ –

0.005…0.01; $MgSO_4$ – 0.5; KH_2PO_4 – 2.0; KCl – 0.5; water – 1.0 L; pH – 3.0. Producer cultivation according to the described proceeding determines the amylolytic activity increase in *Aspergillus niger* 33-19 CNMN FD 02A strain by 23.7-28.9%, as compared to control on the day of maximum biosynthesis, the technologic cycle being reduced with 24-48 hours.

Proceeding II: The basic nutrient medium and cultivation conditions mentioned above are supplemented with $[Co(NioxH)_2(Sam)_2][TiF_6]\cdot 3H_2O$ coordination compound in the concentration of 5.0 mg/L. The proceeding advantage consists in the increase of acid labile and acid stable amylase activity by 64 and 48% respectively, towards the control variant on the day of maximum biosynthesis, and in the reduction of the technologic cycle with 24 hours.

Proceeding III: The basic nutrient medium and cultivation conditions mentioned in proceeding I are supplemented with $[Co(NioxH)_2(An)_2]_2[TiF_6]\cdot 3H_2O$ coordination compound in the concentration of 1.0 mg/L. The advantage of this proceeding consists in increasing acid labile and acid stable amylase activity by 40 and 29%, as compared to the control sample on the day of maximum biosynthesis, and in reducing the technologic cycle with 24 hours.

The full scheme of performing the proceedings of amylase oriented synthesis by *Aspergillus niger* 33-19 CNMN FD 02A micromycete in submerse culture is exposed in Fig. IV.4.

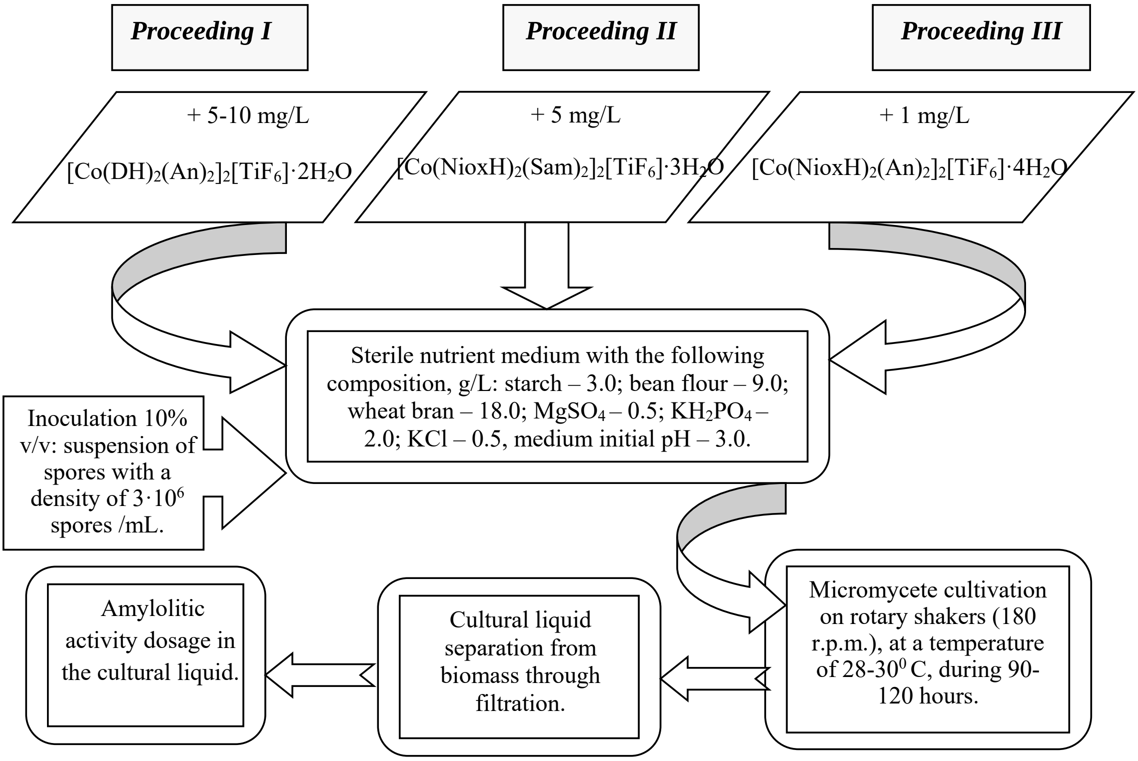


Fig. IV.4. The scheme of performing the proceedings of amylase oriented synthesis by *Aspergillus niger* 33-19 CNMN FD 02A micromycete with chemical stimulators application [62].

A. Proceedings of proteolytic enzymes microbial oriented synthesis at submerse cultivation of fungal origin producers with the application of chemical regulators.

B.1. Proceedings of protease oriented synthesis at submerse cultivation of *Trichoderma koningii* Oudemans CNMN FD 15 micromycete strain with the application of chemical regulators [39, 40, 70].

The results of a series of researches confirm the beneficial influence, with various aspects, of cobalt, copper and zinc coordination compounds based on oximes on the activity of the protease complex, synthesized by *Trichoderma koningii* Oudemans CNMN micromycete strain [21, 22, 46, 50, 59, 65], marking as efficient biostimulators the binuclear compounds of cobalt with dimethylglyoxime and selenocarbamide – $[Co(DH)_2(Seu)_{7/4}(Se\text{-}Seu)_{1/4}]_2[TiF_6]\cdot H_2O$, copper with disulfanilamide glyoxime – $[Cu(DSamH_2)_3]SO_4$, and zinc with 4,4-bipyridyl- $[Zn_2(CH_3COO)_4(NioxH_2)_2bpy(H_2O)_2]$.

Considering the selected compounds, there have been developed three proceedings of protease oriented synthesis, using as a producer the micromycete strain *Trichoderma koningii* Oudemans CNMN FD 15 in submerse culture.

Proceedings description: *Trichoderma koningii* Oudemans CNMN FD 15 strain is cultivated during 8-9 days on the basic nutrient medium with the following composition, g/L: wheat bran – 20.0; soy flour – 10.0, $(NH_4)_2SO4$ – 1.0; $CaCO_3$ – 2.0; pH – 6.25; supplemented with coordination compounds as follows:

Proceeding I: $[Co(DH)_2(Seu)_{7/4}(Se\text{-}Seu)_{1/4}]_2[TiF_6]\cdot H_2O$ coordination compound in the concentration of 10 mg/L and 15 mg/L.

Advantage: it increases the biosynthesis of acid protease by 122.0-37.1%, and neutral protease by 13.8-44.1%; it offers the possibility of attaining proteolytic enzyme complexes with a programmed composition.

Proceeding II: $[Cu(DSamH_2)_3]SO_4$ coordination compound in the concentration of 5 mg/L and 10.0 mg/L.

Advantage: it increases the biosynthesis of acid protease by 110.7-121.4% on the 8-th cultivation day, of neutral protease – by 52.5% on the 9-th cultivation day (neutral protease activity is higher to the control variant during the whole cultivation period – on the 9th and 10th cultivation days), fact characterizing it as a specific stimulator of neutral protease in *Trichoderma koningii* Oudemans micromycete; it offers the opportunity to obtain proteolytic enzyme complexes with a programmed composition.

***Proceeding III*: ** $[Zn_2(CH_3COO)_4(NioxH_2)_2bpy(H_2O)_2]$ coordination compound in the concentration of 5 mg/L.

Advantage: It increases the biosynthesis of acid protease by 110.2%, and that of neutral protease by 69.7% on the 8-th cultivation day; the technologic cycle being reduced with 24 hours.

B.2. Proceedings of proteolytic enzymes oriented synthesis at submerse cultivation of *Fusarium gibbosum* CNMN FD 12 strain with the application of Zn(II) coordination compound with oximes [64].

The cultivation of Fusarium gibbosum CNMN FD 12 micromycete in the presence of Zn(II) coordination compound based on 1,2-cyclohexanedione dioxime with a differentiated influence on the enzyme complex components, was performed according to the proceeding exposed in Fig. IV.5.

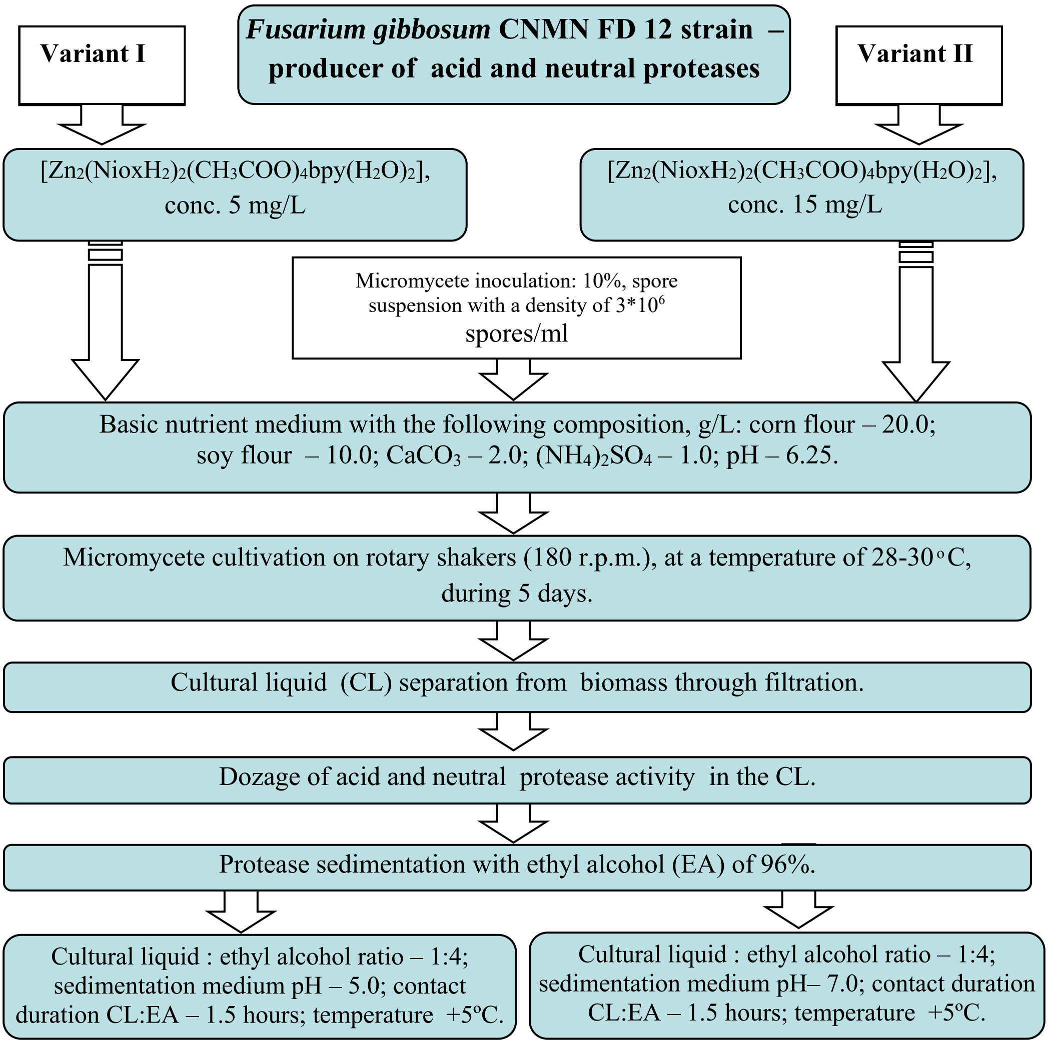


Fig. IV.5. The scheme of performing the cultivation proceeding of *Fusarium gibbosum* micromycete in the presence of $[Zn_2(NioxH_2)_2(CH_3COO)_4bpy(H_2O)_2]$ coordination compound

The coordination compound included in the producer strain culture medium, in the concentration of 5.0 mg/L, determines the increase of acid protease activity (variant I), while in the concentration of 15.0 mg/L – it ensures the increase of neutral protease activity (variant II). Varying the type, the coordination compound concentration and the duration of micromycete cultivation, it is possible to obtain, depending on the application domain, enzyme preparations with a balanced share of neutral and acid preparations, or preparations containing preferentially neutral protease.

According to the presented scheme, the producer strain is cultivated according to the proceedings of directed cultivation (presented in alternative variants), after different purposes – acid and neutral protease attainment.

B.3. Proceedings of proteolytic enzyme oriented synthesis at submerse cultivation of *Fusarium gibbosum* CNMN FD 12 strain in the presence of $[Co(DH)_2(Thio)_2]_3F[SiF_6]\cdot 1.5H_2O$ coordination compound [71].

The proceeding stipulates the in depth cultivation of *Fusarium gibbosum* CNMN FD 12 micromycete strain, during 5 days, on basic nutrient medium with the following composition g/L: cornflour – 20.0; soy flour – 10.0; $CaCO_3$ – 2.0; $(NH_4)_2SO_4$ – 1.0; pH – 6.25, supplemented with $[Co(DH)_2(Thio)_2]_3F[SiF_6]$ $1.5H_2O$ coordination compound in the concentration of 0.0010…0.0015 g/L. Micromycete cultivation is performed in conditions of permanent shaking (180 r.p.m.) at a temperature of 28-30° C.

Advantage: the increase of neutral protease activity by about 42.6-59.0%; the reduction of cultivation duration with 24 hours.

B.4. Proceedings of proteolytic enzyme oriented synthesis at submerse cultivation of *Fusarium gibbosum* CNMN FD 12 strain in the presence of regulators of chemical origin [64-66].

It has been found out that oxime based metal complexes, different by complex generating metal, type of oximes and supplementary ligands or/and composition of anions from the external sphere act as stimulators and regulators of the proteolytic complex synthesized by *Fusarium gibbosum* CNMN FD 12 strain by ensuring enzyme activity and yield increase, culture development intensification by reducing cycle duration and synthesis preferential orientation to a certain or another component of the enzyme complex. The obtained data served as a basis for developing some proceedings of oriented synthesis, according to the scheme presented in Fig. IV.6.

The proceedings of micromycete cultivation with coordination compounds presence can be applied for the following purposes:

I – attainment of proteolytic enzyme preparations, with high activity and balanced structure of neutral and acid proteases.

II – attainment of proteolytic enzyme preparations, with high activity, enriched with neutral proteases;

III – attainment of proteolytic enzyme preparations, with high activity, enriched with neutral proteases in reduced intervals of time.

Proceeding I: *Fusarium gibbosum* CNMN FD 12 strain is cultivated within 5 days on the basic nutrient medium, g/L: cornflour – 20.0; soy flour – 10.0; $CaCO_3$ – 2.0; $(NH_4)_2SO_4$ – 1.0; pH 6.25; supplemented with coordination compounds as follows:

a) $[Cu(DSamH_2)_3]SO_4 \cdot 5H_2O$ coordination compound, in the concentration of 1.0 mg/L.

Advantage: acid protease (70%) and neutral protease (95%) activity increase;

b) $[Co(NioxH)_2(Thio)_2][PF_6]$ coordination compound, in the concentration of 5.0 mg/L.

Advantage: acid protease (60%) and neutral protease (85%) activity increase;

c) $[Co(DH)_2(Seu)_{1.75}(Se\text{-}Seu)_{0.25}]_2[TiF_6] \cdot H_2O$ coordination compound, in the concentration of 10.0 mg/L.

Advantage: acid protease (70%) and neutral protease (70%) activity increase;

Proceeding II: *Fusarium gibbosum* CNMN FD 12 strain is cultivated within 5 days on the basic nutrient medium, g/L: cornflour – 20.0; soy flour – 10.0; $CaCO_3$ – 2.0; $(NH_4)_2SO_4$ – 1.0; pH 6.25; supplemented with coordination compounds as follows:

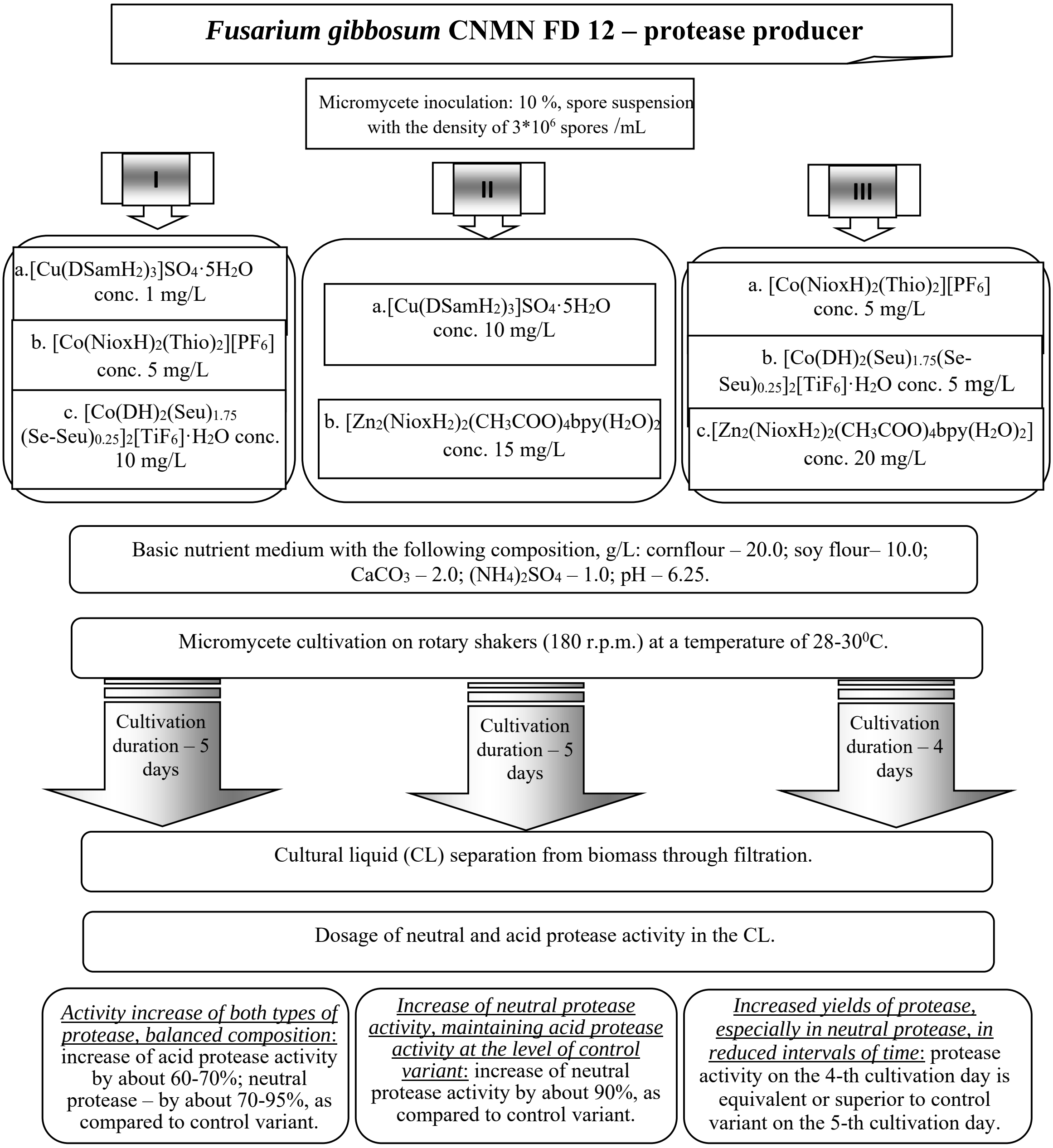
a) $[Cu(DSamH_2)_3]SO_4 \cdot 5H_2O$ coordination compound, in the concentration of 10.0 mg/L.

Advantage: the activity of acid protease is equivalent to that of control variant; neutral protease activity increases by 80%;

b)$[Zn_2(NioxH_2)_2(CH_3COO)_4bpy(H_2O)_2]$ coordination compound, in the concentration of 15.0 mg/L.

Advantage: the activity of acid protease is equivalent to that of control variant; neutral protease activity increases by 90%;

The sheme of performing the cultivation proceedings of *Fusarium gibbosum* micromycete is exposed in Fig. IV.6.



***Fusarium gibbosum* CNMN FD 12 – protease producer**

Micromycete inoculation: 10 %, spore suspension with the density of $3*10^6$ spores /mL

I

a.$[Cu(DSamH_2)_3]SO_4 \cdot 5H_2O$ conc. 1 mg/L

b. $[Co(NioxH)_2(Thio)_2][PF_6]$ conc. 5 mg/L

c. $[Co(DH)_2(Seu)_{1.75}(Se\text{-}Seu)_{0.25}]_2[TiF_6] \cdot H_2O$ conc. 10 mg/L

II

a.$[Cu(DSamH_2)_3]SO_4 \cdot 5H_2O$ conc. 10 mg/L

b. $[Zn_2(NioxH_2)_2(CH_3COO)_4bpy(H_2O)_2]$ conc. 15 mg/L

III

a. $[Co(NioxH)_2(Thio)_2][PF_6]$ conc. 5 mg/L

b. $[Co(DH)_2(Seu)_{1.75}(Se\text{-}Seu)_{0.25}]_2[TiF_6] \cdot H_2O$ conc. 5 mg/L

c.$[Zn_2(NioxH_2)_2(CH_3COO)_4bpy(H_2O)_2]$ conc. 20 mg/L

Basic nutrient medium with the following composition, g/L: cornflour – 20.0; soy flour– 10.0; $CaCO_3$ – 2.0; $(NH_4)_2SO_4$ – 1.0; pH – 6.25.

Micromycete cultivation on rotary shakers (180 r.p.m.) at a temperature of 28-30^{0}C.

Cultivation duration – 5 days

Cultivation duration – 5 days

Cultivation duration – 4 days

Cultural liquid (CL) separation from biomass through filtration.

Dosage of neutral and acid protease activity in the CL.

Activity increase of both types of protease, balanced composition: increase of acid protease activity by about 60-70%; neutral protease – by about 70-95%, as compared to control variant.

Increase of neutral protease activity, maintaining acid protease activity at the level of control variant: increase of neutral protease activity by about 90%, as compared to control variant.

Increased yields of protease, especially in neutral protease, in reduced intervals of time: protease activity on the 4-th cultivation day is equivalent or superior to control variant on the 5-th cultivation day.

Fig. IV.6. The scheme of performing the cultivation of *Fusarium gibbosum* micromycete in the presence of regulators of chemical origin [65].

C. Proceedings of lipase oriented synthesis with *Aspergillus niger* 412 CNMN FD 01 strain.

According to the previous research, it has been established that Co coordination compounds with oximes and a resembling structure exert a beneficial effect on the process of hydrolase synthesis in different species of micromycetes.

Thus, $[Co(MH)_2(Py)_2][BF_4]\cdot H_2O$ compound was selected as a stimulator of *A. niger* 412 strain lipolytic activity. The high stimulating effect exerted by the metal complex on lipase biosynthesis in the strain served as a basis for developing the proceeding of lipase oriented microbial synthesis using as a producer the *Aspergillus niger* 412 CNMN FD 01 micromycete strain.

The proceeding stipulates deep cultivation of *Aspergillus niger* 412 CNMN FD 01 culture, within directed conditions, on the basic nutrient medium, supplemented with $[Co(MH)_2(Py)_2][BF_4]\cdot H_2O$ bio stimulating coordination compound in a concentration of 5 to 20 mg/L medium. Cultivation duration – 5 days.

The proceeding ensures the increase of producer lipase biosynthesis by 31.2-49.9%, under the applied concentration.

D. Proceedings of pectolytic enzyme oriented synthesis with *Rhizopus arrhizus* Fischer C strain [29, 34, 70].

The technology includes the directed submerse cultivation of *Rhizopus arrhizus* Fischer, on accessible nutrient media, and the pectolytic enzyme complex recuperation from the strain cultural liquid through sedimentation with alcohol.

As biostimulators, there can be alternatively used: $[Co(DH)_2(Thio)_2]_2[SiF_6]\cdot 3H_2O$, $[Co(DH)_2(Thio)_2]_3F[SiF_6]\cdot 1.5H_2O$ compounds in concentrations of 5-10 mg/L.

Proceeding description: *Rhizopus arrhizus* Fischer C strain is cultivated during 48-72 hours on the basic nutrient medium composed of g/L: beet pulp – 22...23 (or 98,8 mL beet pulp extract: 45 g/L [18]); cornflour – 14...16; glucose – 0.9-1.1; $MgSO_4$ – 0.6-0.8; $(NH_4)_2SO_4$ – 0.9-1.1; $ZnSO_4$ – 0.24...0.26; pH 4.5-4.7, supplemented with $[Co(DH)_2(Thio)_2]_2[SiF_6]\cdot 3H_2O$,

$[Co(DH)_2(Thio)_2]_3F[SiF_6]\cdot 1.5H_2O$ coordination compounds in concentrations of 5-10 mg/L. Cultivation is performed in conditions of permanent shaking (180-200 r.p.m.) at a temperature of 28-30° C.

Advantages: increase of producer pectolytic activity by 143-184%; technologic cycle reduction with 24 hours.

The originality of the technology developed on the basis of the given proceeding consists in: 1 – using a new selected pectinase producer – the strain of *Rhizopus arrhizus* Fischer mycelial fungi with a high and stable capacity of pectinase synthesis and short development cycle (48-72 hours), 2 – using Co(III) coordination compounds with dimethylglyoxime as biostimulators and stabilizers of biosynthetic capacity.

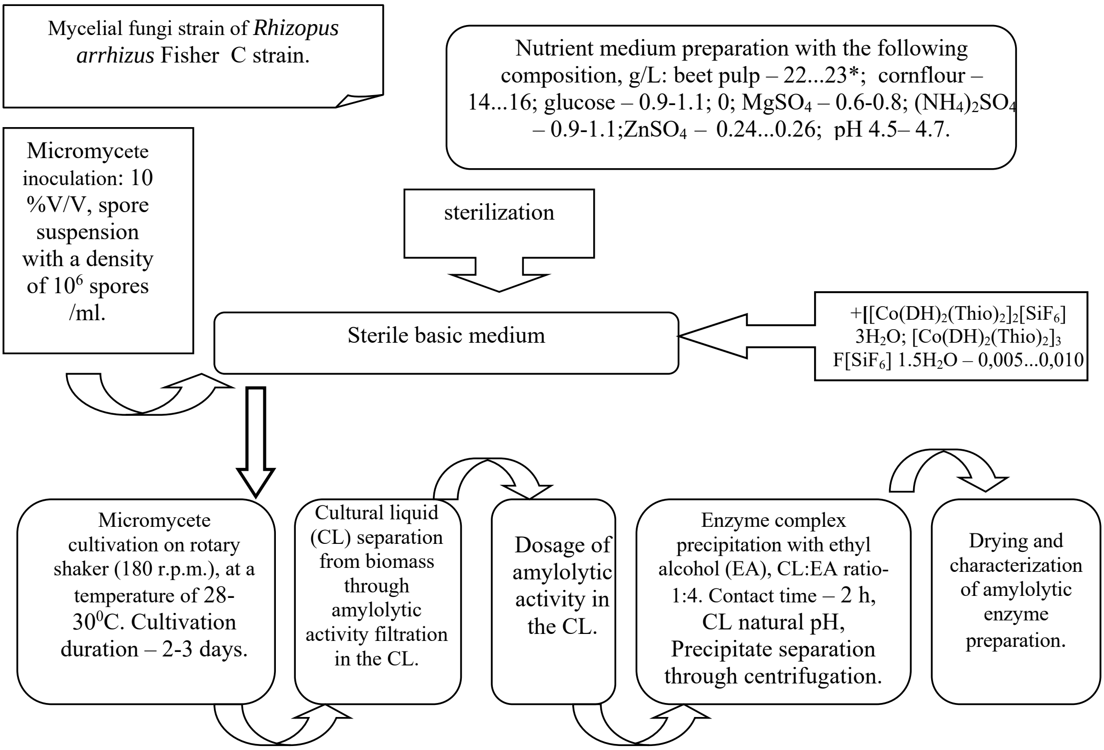


Fig. IV.7. Technologic flow scheme of pectolytic enzymes attainment through oriented synthesis at in depth cultivation of *Rhizopus arrhizus* Fischer C strain micromycete.

The development of environment harmless proceedings of microbial hydrolase oriented synthesis aim to involve stable producer strains, cheap nutrient media based on food industry subproducts; and to use coordination compounds as factors directing the biosynthetic processes, thus contributing to the growth of competitive hydrolytic enzyme production, offering opportunities for diversifying enzyme preparations, and ensuring the development of innovational biotechnologies aimed at producing microbial bioactive principles with increased efficiency.

Conclusion: The compounds of some transition metals based on α-dioximes manifest different useful properties: as catalysts in technological processes of dyeing fabrics in the textile industry; catalysts of different chemical reactions in the chemical industry, including double function properties as catalysts and stabilizers within reactions of producing polyurethanes; inhibitors of steel corrosion processes in aquatic medium; materials with well-expressed dielectric properties etc. The described compounds can be practically used in different fields of economy, such as: textile and chemical industries, urban household for protecting and prolonging exploitation term of closed systems, having water as an agent, in electronic industry etc.

According to the results of biological researches exposed in the present monograph, coordination compounds of transition metals based on oxime ligands have great application perspectives in microbial biotechnology for purposes of increasing production and diversifying valuable biologically active substances.

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