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Ecological Aspects of Plant Growth Regulator Use

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Abstract: The plant growth regulators (ivin, emistim C, agrostimulin) stimulate the growth of soil microorganism natural associations, initiate synthesis of biologically active substances by microorganisms, intensify the ability of microbial community to produce antibiotics to phytopathogenic bacteria. The use of the plant growth regulators and the plant chemical protectors (phenpiklonil, phludioxonil, diphenoconazol) in combination decreases the negative influence of fungicides on plants and microflora, raises the number of microbes, resistant to pesticides, and increases the activity of microbial redox system capable to initiate an oxidizing destruction of pesticide dangerous for environment.
Key words: Plant growth regulators, fungicides, soil microflora, environment.

Introduction

Actual problem of present agricultural production is the development of technologies, which promote rise of plant productivity and at the same time are ecologically safe for an environment and health of the men.

The considerable attention is given to the plant growth regulators (PGR) in the economically developed countries. They are used for stimulation of seed germination, activation of plant vegetative growth, acceleration of flowering and maturing, rises of plant productivity, protection of plants against some diseases, etc.

The complex investigation of plant growth regulators are carried out in the Institute of Bioorganic Chemistry and Oil Chemisry of the National Academy of

Sciences of Ukraine together with the experts of other scientific. Fulfiled study consisted some stages - from creation and comprehensive study to elaboration of technologies of their use in agricultural production. Detailed researches of the physical-chemical and physiological properties, and check-up the action of more than 100 created preparations connected with 24 agricultural plants have allowed to create some individual preparations on the base of N-oxide pyridine derivates and their various combinations with plant growth regulators of a natural origin. The obtained data testify to high effectiveness of new PGR both for treatment of seed, and for spraying crops (Ponomarenko et al.,1999).

The important aspect of the PGR action is their ability to raise of plant resistance to diseases. The use PGR together with plants chemical protectors allowe to remove phytotoxic effect and also to reduce the load of pesticides on plants.

For creation the effective ecologically safe PGR and their combination with plant chemical protectors it is necessary to study their influence not only on plants, but also on the main component of edatopes - soil microorganisms. It is known, that the soil microbes play leading role in the biogenic element cycle, synthesis of biologically active substances, humus transformation, etc. Therefore it is logical to raise the question about what influence have the PGR on soil microflora, on mutual relation between microbes and plants, on forming and functioning of microbial soil communities. It is known that some cultures do not react upon biostimulators in a medium, others react positive and this correlates with concentration of PGR and composition of a medium (Greenberg, Tirpak, 1960; Skalka et al., 1981).

There are a little data of phytohormone influence on soil microbial communities. It was marked the absence of gibberelin influence on the number of microorganisms and activity of their respiration in soil under experiment (Lu, Bollen, 1968). It was shown, that auxin- and citokinine-like substances do not influence on pure cultures of nitrogenfixing microorganisms. However they raise the activity of associative nitrogen fixation by stimulating the root system development (Volkogon, 1996).

The preparations which were synthesized on the basis of N-oxides pyridine derivates and their combination with natural plant growth regulators have unique phisical-chemical properties: its are very sensitive to slow mechanical oscillatons, change of magnetic and electrical field, infrasound. Some of investigated compounds have exhibited properties of electron or ionic semiconductors, that makes their capable to execute funtions of the communications in live organismes - transmission of electrical, magnetic impulses and other signal information (Ponomarenko et al., 1999).

Some aspects of their influence on plant organisms are studied, but data concerning influence on soil microorganisms are absent. The studying new plant growth regulators and their combination with pesticides influence on microbial communities was the purpose of our researches.

Materials and Methods

There were investigated such plant growth regulators : ivin (N-oxide 2,6-dimethyl-pyridine), emistim C (produced by *Cylindrocarpon magnesianum IMBF 10004* is the complex of natural growth substances - phytohormone analogs of auxine, cytokinine, gibberelline nature, saturated and unsaturated fat acids, amino acids, polysaccharides and microelements), agrostimulin (the composition containing 97% of emistim C and 3% ivin.There were investigated also the corporation "Novartis" fungicides: phenpiklonil (for presowing treatment of spring and winter wheat and barley against coverd smut, snow mould, and root rots); phludioxonil (for treatment of corn against root and stem rots); diphenoconazol (for treatment of grain cultures against *Tilletia, Cochliobolus, Septoria, Ustilago, Pyrenophora* - agents of plant deseases).

The investigation influence of plant growth regulators and fungicides on growth parameters of the natural soil microbe associations carried out under batch cultivation on the synthetic mineral medium of such composition ($g \cdot l^{-1}$): $NaNO_3$ - 2.0; K_2HPO_4 - 2.0; $MgSO_4$ -0.5; KCl -0.5; glucose - 5.0. The concentration of PGR and fungicides were used such as usually use in agriculture: emistim C $2 \cdot 10^{-4}$, ivin $3 \cdot 10^{-5}$, phenpiklonil $6 \cdot 10^{-5}$, phludioxonil $2 \cdot 10^{-4}$, diphenoconazol $4 \cdot 10^{-4}$. Control and experiment variants sowed by 50 ml of chernozem water suspension (dilution 1:1000) to 1000 ml of the medium. Incubation of test tubes carried out at 28^0C under shaking. Microbial biomass was determined by optical density. The general accepted formulas for calculations of associations growth were used (Pirt, 1975).

The quantity of fungicide resistant microorganisms determined after 48 h cultivation. For this purpose 1 ml of cell suspension plated on the solid medium of the above mentioned composition with 0,02g fungicides (by Carbon) per 1000 ml. The microorganism biomass determined in the suspension at the same time.

The oxidation-reduction fermentative systems of living microbial cells characterized by assotiation ability to oxydize hydrogen peroxide. For this purpose 2 ml of 48-h microbial suspension mixed with 5 ml of 2 % hydrogen peroxide and standed at temperature of melting ice during 2.5 h. Controls were: sterilized microbial suspension and H_2O_2 without suspension. After 2.5 h the reaction was stopped by addition 5ml of 10% sulfuric acid. The residual amount of hydrogen peroxide determined by titration with 0.1N permanganate potassium. Activity of H_2O_2 determined by difference between experiment and control data with the subsequent calculation per 100 mg of biomass for 1 h.

Fungicide activity studied on plates with phytopathogenic micromycetes as tests-cultures: *Fusarium oxysporum (Schlechtendal 1824), Snyder et Yansen 1940 N 54620; F. oxysporumm N 55759; F.avenaceum (Corda 1837), Fries 1949, N 55403; F.giblosum (Appel et Wollenweber 1910), Bilai 1955 N 50053.* All of them were received in the department of physiology and systematic of micromycetes of Institute of Microbiology and Virology National Academy of Sciences of Ukraine. After 48h incubation the bacterial cells separated by a centrifuging, the filter paper disks impregnated with cultural liquid and laid out them on the plates with newly-sowed phytopathogenic micromycetes.

This method also was used for studying of association ability to depress the phytopathogenic bacteria: *Pseudomonas syringae (van Hall 1902), Bergey et*

al. 1930) 8511, P. fluorescens (Migula 1895) N 8573, Ervinia carotovora subsp. carotovora (Jones 1901), Bergey et al. 1923)N 8982, Xanthomonas campestris pv. campestris (Pammel 1895), Dowson 1939 N 80036, Clavibacter michiganensis subsp michiganensis (Smith 1910), Davis et al. 1984 N 13a, Agrobacterium tumefaciens (Smith et Townsend 1907), Conn 1942 N 8628. All of them were received in the department of phytopathogenic bacteria of Institute of Microbiology and Virology National Academy of Sciences of Ukraine.

Results and Discussion

Obtained data have shown that PGR as additional components to medium directly influence on growth parameters of soil microbial natural associations. They raise the specific growth rate, increase the number of generations, shorten the lag-phase and cell doubling time (tab.1).

Tab. 1. Influence of PGR and their Combination with Fungicides on the Growth of Soil Microorganism Natural Associations

Variant of experiment	Specific growth rate, h^{-1}	Cell doubling time, h	Number of generations at 6 h	Lag-phase, h
Control	0,18	3,9	1,5	22,5
Emistim C	0,22	3,2	1,9	20,0
Ivin	0,22	3,2	1,9	14,0
Phenpiklonil	0,09	7,7	0,8	24,0
Phludioxonil	0,17	4,1	1,5	17,0
Diphenoconazol	008	8,7	1,2	26,2
Emistim C+ Phenpiklonil	0,20	3,5	1,7	18,5
Emistim + Phludioxonil	0,30	2,3	2,6	18,5
Emistim C +Diphenoconazol	0,24	2,9	2,1	20,0
Ivin + Phenpiklonil	0,27	2,6	2,3	21,2
Ivin + Phludioxonil	0,30	2,3	2,6	18,0
Ivin+Diphenoconazol	0,28	2,5	2,4	19,6
Agrostimulin	0,35	2,0	3,0	18,0
Agrostimulin + Phenpiklonil	0,32	2,2	2,8	18,0
Agrostimulin + Phludioxonil	0,32	2,2	2,8	18,5
Agrostimulin+Diphenoconazol	0,29	2,4	2,5	18,0

The presence of fungicides in medium oppresses the microorganism growth; their combination with PGR decrease this negative influences and save the positive action of PGR.

The use of phytohormones and fungicides in agricultural production raise the question about stability these substances in microorganism presence. It is known that soil microbes are capable to synthesize enzymes, such as oxydases and hydrolases, which catalyze destruction of fungicides. However the transformation of plant growth regulators in soil is not enough investigated.

Obtained data testify that ivin or emistim C stimulate the germination of tests-plant seed under development of natural microbial associations in presence

of these compounds. So, the cultural liquid after 48h of microbe cultuvation together with PGR intensified the stimulation of radish growth by 18-43% in comparison with the action of Ivin or Emistim alone. It is possible that PGR stimulate the microbe production of additional phytogormone-like substances wich are able to intensify the PGR action.

The similar researches were carried out with fungicides. Phenpiklonil and phludioxonil, in concentrations which are usually used in agriculture production, oppressed the test - plants by 18.1-27.2 % in comparison with control. Their combination with PGR took away the toxic influence of fungicides, just as the Emistim positive action was decreased a little and ivin - did not vary essentially.

It is known that negative influence of pesticides and products of their transformation on environment is stipulated by their toxic properties and ability to accumulation in soil and plants. At the same time there are a lot of soil microorganisms which are resistant to pesticides and even able to their destruction (Cook,1987; Hund, Schek, 1994). The speed and degree of pesticide degradation in soil are depended on protective adaptation and biochemical activity of microorganisms.

Our researches have shown that PGR promoted increasing the number of microorganisms, resistant to fungicides and capable to their destruction (Fig.1). As is obvious from the data, it is not observed appearance of microbes adapted to definite sort of fungicide in microbial community. It is possible that the increase the number of microorganisms, resistant to fungicides, is happened owing to activation the processes of energetic and constructive metabolism by PGR that raises microbial stability to the unfavorable factors including fungicides.

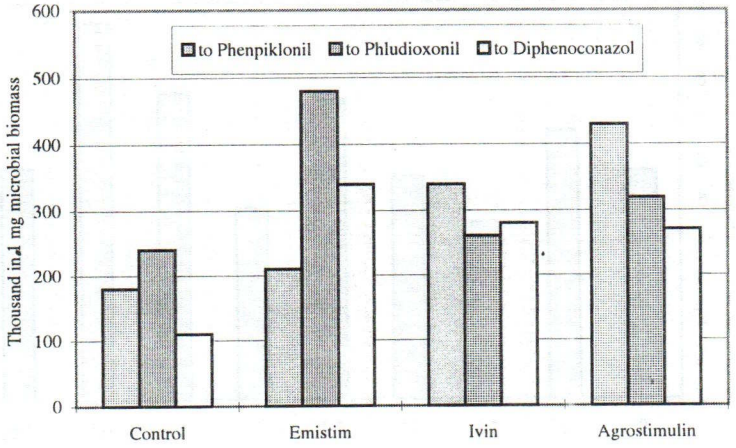

Fig.1. Plant growth regulator influence on the quantity of microbes resistant to Fungicides

It is universally recognized the leading role of microbial redox system at the initial stages of pesticides destruction (Alexander, 1960; Cook, 1987).

The multipurpose oxygenases begin oxidize the periphery part of these substance molecules (Hayaiski, 1974). At the first stages the pesticides destruction the microbe redox systems which realize oxidation and hydroxylation of these substrata destroy their cyclic structure (Cook, 1987; Hayaiski, 1974). We researched one of the most spread oxidation-reduction enzymes - catalase. It was shown that plant growth regulators, fungicides and their combination as medium components essentially influence on the decomposition of hydrogen peroxide by natural associations of soil microorganisms (Fig. 2).

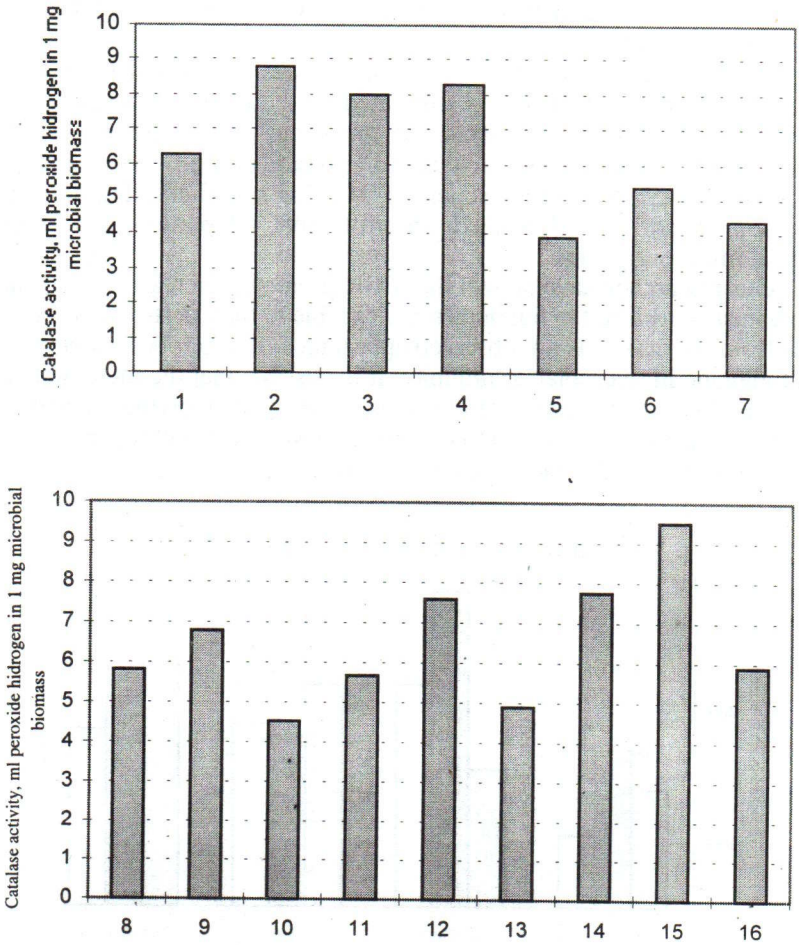

Fig.2. Influence of plant growth regulators and fungicides on the catalase activity of soil microbe associations1-control; further under addition in medium: 2- emistim C, 3- ivin, 4-agrostimulin, 5- phenpiklonil, 6- phludioxonil, 7- diphenoconazol, 8-emistim C + Phenpiklonil, 9-emistim C + phludioxonil, 10-emistim C + diphenoconazol, 11-ivin+phenpiklonil, 12-ivin+phludioxonil, 13- ivin+diphenoconazol, 14- agrostimulin+phenpiklonil,

Emistim C, ivin and agrostimulin stimulate catalase activity of microorganisms by 27.3-40.1 % in comparison with microbial activity in the control. The activity of H_2O_2 decomposition was decreased by 15.1-38.3 % at the presence of fungicides. Phenpiklonil had the greatest toxicity effect, phludioxonil - the least toxicity effect. The application of PGR together with fungicides decreased their negative influence. The greatest toxicity effect was marked in the presence of phludioxonil and somewhat less - in variants with diphenoconazol and phenpiklonil.

The rise of oxidation-reduction activity of associations allows to suggest that potential ability of microbial communities to destruction of fungicides is increased in the presence of PGR. The discovered fact testifies to positive role PGR in purification of an environment from pesticides.

Detected specificity in development of pesticide biodestructors is closely bounded with the problem of fungicide activity concervation under the action of microorganisms.

Our researches of antifungal activity of studied fungicides testify that all preparations oppressed the development of phytopathogenic micromycetes. At dilution of fungicide solutions essentially reduced antifungal activity on the whole; it was marked only in relation to separate cultures. So, phenpiklonil delayed the growth of *F. avenaceum* 55403 under concentrations $6 \cdot 10^{-2}$ and $6 \cdot 10^{-3}$; phludioxonil delayed the growth of the *F. giblosum* 50053 under concentrations $2 \cdot 10^{-2}$ and $2 \cdot 10^{-3}$; diphenoconazol oppressed the development of *F. oxysporum* 54620 under concentration $4 \cdot 10^{-3}$. All researched preparations were not effective under concentrations recommended to agriculturale production.

On the other hand it was found that phenpiklonil, phludioxonil and diphenoconazol are nonresistant to microbial destruction. Cultural liquid of 48 h microbial associations on the medium containing effective concentrations of fungicides did not oppress the test-culture growth that is possible was connected with their partial or complete destruction.

It is known, that PGR increase the plant resistant on the whole. It was interesting to study the action of PGR, fungicides and their combinations on phytopathogenic bacteria. It was shown that the preparations themself did not revealed the antibiotic activity to test-cultures. But antagonistic action to some phytopathogenic bacteria was revealed at separate variants of 48h microbial associations which were grown in the presence of PGR. So, the associations, which were developed under control medium oppressed only *X.campestris* 8003b and *A.tumefaciens* 8628. The presence of emistim C enlarged the spectrum of sensitive bacteria: there were oppressed four test-cultures - *X. campestris* 8003b, *A.tumefaciens* 8628, *P. fluorescens* 8573 and *P.syringae* 8511. The microbial associations which grow at the presence of compositions emistim C + phludioxonil, emistim C +diphenoconazol, agrostimulin + phludioxonil, agrostimulin + diphenoconazol revealed the antibacterial activity too.

The obtained data allow to state the supposition that although emistim C, ivin and agrostimulin do not have antibiotic activity to phytopathogenic bacteria, but they favour development of separate antagonists to the agents of plant deseases which are memers of microbial communities. This supposition was confirmed by learning of soil antibiotic activity with use of PGR in field experiments. The soil antibiotic potential was found higher in comparison with

the same of microbial associations culture liquids. It is possible that antibiotic substances in soil are adsorbed by soil organic matter including humus that saves their stability and promotes rise of biochemical and biological activity. Soil antibiotic potential under use of plant growth regulators was higher than under control and appeared in relation to such phytopathogenic bacteria: *P.syringae 8511, P. fluorescens 8573, E. carotovora 8982, X.campestris 8003b, C.michiganensis 13a, A.tumefaciens 8628.*

Thus, the fulfiled researches have shown that ecological aspect of application the plant growth regulators (ivin, emistim C, agrostimulin) in connection of their influence on soil microflora is very important. It consists in the PGR ability to activate the growth of soil microbial communities and synthesis biologically active compounds including antibiotic substances to phytopathogenic bacteria. That promotes improvement phytosanitary conditions in soil as a result.

The combined use of plant growth regulators with substances of chemical plant protection decreases the negative fungicides influence on plants and soil microflora. At the same time it raises the number of microorganisms resistant to xenobiotics, increases the activity of redox systems which initiate the destruction of these dangerous to an environment compounds.

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EKOLOŠKI ASPEKTI REGULACIJE RAZVOJA BILJAKA

- originalni naučni rad-

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Rezime

Regulatori biljnog razvoja (ivin, emistim C, agrostimulin) stimulišu razvoj prirodnih asocijacija zemljišnih mikroorganizama, podstiču sintezu biološki značajnih grupa mikroorganizmima i jačaju sposobnost mikrobiološke produkcije antibiotika koji će sprečiti razvoj fitopatogenih bakterija. Primena regulatora biljnog razvoja i kombinovanih zaštitnih sredstava biljaka (fenpiklonil, fludioksinol, difenoconazol) smanjuju negativni uticaj fungicida na biljke i mikrofloru, povećavaju broj mikroba otpornih na pesticide, i povećavaju aktivnost mikrobiološkog redoks sistema sposobnog da potakne razgradnju pesticida opasnih po životnu sredinu.