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P R I M E R

– Review of application and achieved effects–

CONTENT

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Allelopathy and Agrostemin

A Swiss botanist M. A. P. de Candale (1832) introduced the term allelopathy as biological discipline, which referred to plant interactions, in other words biochemical influence of substances produced by plants. Many years later, Molish (1937) used the term allelopathy to include both negative and positive biochemical interactions which include microorganisms as well. This definition was later accepted by many scientists and as such has been in use in scientific literature until today.

In the last few decades scientific research in the field of allelopathy have intensified, primarily the study of biochemical interactions between plants in natural and artificial ecosystems.

Allelopathy as a multidisciplinary science which studies biochemical interactions between plants is increasingly applied in contemporary production worldwide and in terms of the increase in yield and quality of cultivated plant species on allelopathic basis it is established on both positive and negative allelopathy as fundamental directions of allelopathic interaction of plants.

Namely, negative allelopathy which deals with biochemical interactions in which one plant species can excrete substances that inhibit the growth and development of other plants without suffering any inhibition itself can find its practical application as a principle in contemporary agricultural practice and biological fight against weed. This has been suggested by the results of American researchers, Rice (1) (2) as well as Putman and Duke (3). During this process the plant inhibitory compounds excreted to the environment may be secretions of plant organs or products of decomposition of dead plants or their parts (in some cases even straw), and they are most often organic acids, alkaloids, aldehydes and glycosides (4).

In addition to this significant fundamental results have been achieved in positive allelopathy by the Soviet researchers (5).

It is particularly worth mentioning the significant success of Yugoslav researchers Danica Gajic, PhD, and associates in the field of fundamental and applied allelopathy (6)(7)(8)(9). At the same time, the fields of negative and positive allelopathy are mutually inseparably connected by the feedback of biological influence of one plant to another from the aspect of benefit to humans.

The studies were conducted in the field of positive allelopathy and the experiments have resulted in several categories of mutual interactions of plant species (crops and weeds) when growing together on various types of soil. Ecologically similar species have been separated (wheat–cockle) in order to obtain a series of various forms of interspecific relationships. As the most important form from the aspect of benefit to humans in the production of biomass it is possible to use the mutual relationship between the species where there is stimulation (wheat–cockle) and where there is inhibition (cockle–wheat). Within the mentioned research other crops have also been studied (corn, sunflower, soybean, and other) and cockle as well as other weed varieties within characteristic combinations of plant communities – phytocoenosis. All studies have shown allelopathic effects positive to crops and unfavourable for weeds. The established allelopathic relations in mixed communities which are useful for crops but harmful for weeds have been transformed in abiotic form into a monoculture of cultivated plants.

Cenological and biochemical relations of a pure crop in macro and micro conditions as well as in strictly controlled conditions have been realised based on the complex of chemical mediators of natural origin marked as allelopatins. These substances are produced as **AGROSTEMIN®** preparation.

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Bioactivation properties of Agrostemin

In the course of the studies it has been established that Agrostemin has bioactivating effect. It is based on two types of activation of ecophysiological and biochemical processes:

1. Activation as a consequence of the lack of living factors, and
2. Activation as a consequence of disturbed quantitative and qualitative scope of substances within a plant.

These two types of activation of ecophysiological and biochemical processes occur as a consequence of higher content of allelopathic compounds in the natural preparation Agrostemin in comparison to crops. This becomes logical considering also the results of chemical analysis of Agrostemin, which suggest the quality of its composition. Namely, chemical analyses have shown that Agrostemin, in addition to organic compounds usually present in traces, considering that the preparation is generated from plant material, also contains and in considerable quantity two very important groups of organic compounds:

I <u>Active complex</u>	a) Free amino acids and derivatives of amino acids
	b) Organic acids and derivatives of organic acids
II <u>Inhibitors (in traces)</u>	a) Derivatives ABA (apcisine acids)
	b) Saturated aliphatic carbon hydrogen and
	c) Cyclic inhibitor ($C_8H_{29}N_3O_7$)

Physiological roles of the stated compounds are rather well known.

Optimum quantities of these compounds in Agrostemin preparation have been given in natural ratio considering the specific biotechnology of manufacturing this preparation from natural raw materials.)

It is worth mentioning also that the production of Agrostemin is based on domestic technology, as well as 100% domestic raw materials, and that its application does not introduce harmful ingredients into plants, soil and water.

In metabolic processes of regulation of cultivated plants growth, based on interspecific allelopathic–chemical effect of Agrostemin on plant–substrate mechanism, and for the purpose of yield increase, the primary starting point was the analysis of value of germination energy, the root length as well as other important physiological parameters significant in heterotrophic manner of nutrition. This is important since it is well known that root growth is integral physiological process and its growth dynamic can be considered methodical instructive feature since it is linked to general multiple exchange of compounds of plants with substrate. Thus, for instance, due to the effect of Agrostemin the total root length and the length of the above ground portion of the plant increase, which is expressed secondarily in the course of subsequent development by phosphorus metabolism and the increase of plant energy level. This happens in terms of increased quantity of ATP during photophosphorylation, as well as the increase of chlorophyll <u>a</u> and chlorophyll <u>b</u> contents respectively as well as the total content of chlorophyll (<u>a</u> + <u>b</u>) in plant leaves (10)(11). When positive results of plant transpiration under the influence of Agrostemin (12) are added to this, it becomes clearer that complete photosynthesis in the plants is more intensive. The ultimately leads to the increase of yield and biomass of the cultivated crops.

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The results of enzyme changes of nitrate reductase activation (13), as well as other positive results among which we point out the increase of easily accessible phosphorus (P_2O_5) in soil following the completion of vegetation (14), and reduced consumption of phosphorus which the plant takes from the solution undoubtedly suggest the activating features of Agrostemin.

Experimental research have shown that the plants respond to Agrostemin systematically, both at the level of organism as an entity and at the level of cell and macromolecule. The plant optimizes its life processes (development, growth, fruit–bearing) which in cultivated plants undoubtedly has positive effect on both quality and quantity of the final yield. The said effect on plants makes Agrostemin an outstanding ecological preparation for plant nutrition very purposeful in husbandry, vegetable growing, fruit growing, wine–growing, flower growing and cultivation of meadows.

It is completely harmless for people, animals (including bees) and environment and does not require any special industrial safety measures of protection (33).

Effects of AGROSTEMIN® application on food grains

Under the influence of **AGROSTEMIN®** germination is quicker and well–balanced, the total root length and the above ground portion of the plant increases, small plants are more resistant to freezing.

Such positive trends are also manifested later on during the entire vegetation cycle through more intense clustering and better tolerance of unfavourable climatic factors (frost, drought).

The increase of protein content in some agricultural crops (wheat, corn, soybean, etc.) as well as the increased content of amino acids, particularly essential amino acids, such as tryptophan, lysine (15)(16) have suggested the possibility of improvement of qualitative and quantitative features of final products by the application of **AGROSTEMIN®**.

These increases of the quantity of the said substances occur as a consequence of the observed effect of **AGROSTEMIN®** on intensifying nucleic acid metabolism in young plants (18), as well as of the effect on the relative representation of DNA in wheat germs (17).

The increase of yield under the influence of **AGROSTEMIN®** (wheat, oats, rye, barley, rice, and so on) depending on the type and ecologic conditions range from 7 to 15%, in other words in absolute amounts from 400 to 600 kg/ha.

In addition to the increase of yield in wheat, it has been established that **AGROSTEMIN®** contributes to the improvement of the quality of grain and subsequently flour in a series of parameters.

The increase of content of raw proteins in food grains is rather significant and amounts to 1.5–2%. The exception is malting barley, in which the content of raw proteins remains at the level of control group or there is tendency of reduction.

These increases of raw protein contents under the influence of **AGROSTEMIN®** reflect subsequently on the quality of products in baking and confectionery industries.

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In addition to the increase of raw protein content, better quality parameters are achieved in hectolitre weight. Absolute weights of 1,000 grains and sedimentation values have as a consequence better rheological test properties (pharinographic and extensographic indicators). In particular, there are expressed differences in rheological properties of water absorption, pharinographic quantitative number and energy.

The results of laboratory baking show that the bread made from wheat flour, when the wheat was treated with AGROSTEMIN®, achieves larger volume of the product and considerably better yield of bread and yield of volume. Also, it cannot be disregarded that bread remains fresh longer (24 hours longer).

Considering the results of laboratory testing, it can be said that grain and flour of food grains treated with AGROSTEMIN® have better quality indicators, which are valued when selling and buying raw materials, in other words, quality class of grains is higher and the properties of food products in baking and confectionary industries are better (7)(19)(20).

Effects of AGROSTEMIN® application on corn

Corn is one of the most important cultivated crops in our country. It is indispensable in human and livestock nutrition, and is particularly used as a raw material in food industry since a large number of products are obtained through its processing.

In the corresponding corn cultivation technology, one of agrotechnical measures is application of AGROSTEMIN® as well. Better yield and quality are achieved after treating corn with AGROSTEMIN®, which are the result of intensifying a number of processes going on both in the entire plant and its cells and macromolecules.

In metabolic processes the starting point was the analysis of germination energy values, where an increase of germination energy and root length has been established, from physiological point of view, such root growth dynamics means better exchange of substances in the plant–soil system. Based on more intensive exchange of substances there is faster germination and development of the above ground portion of the plant. Growth of the stem and leaves as well as darker green colour of leaves and stem are observed, which tells us about intensified photosynthesis process, and experimental results have also suggested this. It has been observed from these results that there is an increase of chlorophyll a and b contents in the plants, as well as their total content (a + b). AGROSTEMIN® has positive effect on RNA and protein syntheses in corn hybrids, since it accelerates the synthesis of enzymes, nitrate reductase and ribonuclease in corn (13)(21)(22). Effect of AGROSTEMIN® on content and structure of proteins has also been established. Increase of amino acids has been determined, primarily of essential amino acids (tryptophan, lysine), as well as influence on the content of lipids and ATP–ase activity in plasma membranes and cell mitochondria, which suggests more intensive respiration.

The stated effects suggest positive effects of AGROSTEMIN® on basic physiological and chemical processes which are going on in corn plants, therefore it is quite understandable that the ultimate effect is in increased grain and biomass yield in corn.

This means that in corn treated with AGROSTEMIN® the grain yield is about 400–1000 kg/ha larger in comparison with the grain yield in corn plants not treated with AGROSTEMIN® (25).

Effects of AGROSTEMIN® application on soybean

Basic effects of AGROSTEMIN® on soybean reflect in higher grain yield and improvement of soybean quality, which reflects then in higher content of raw oil and protein, as well as higher resistance to diseases (barberry rust, for instance), pests (mites and ticks, for instance) and unfavourable climatic conditions.

Soybean *(Glicyne max sp.)* is classified in the family of *Leguminosae.* It is an annual herbaceous and leguminous plant. At the depth of 10–20 cm below the soil surface root nodules are formed on which rhizobia nitrogen fixing bacteria – live in symbiosis with soybean, which provide soybean plants with necessary nitrogen.

Depending on the variety, conditions of growing and agrotechnical measures, soybean seeds contain certain percentage of water, protein, fat, cellulose, minerals (Ca, Mg, Zn, J, Mo, etc.), as well as vitamins (A, B, C, K). Due to high protein content in seeds, soybean is very important cultivated plant from the aspect of human consumption, since mostly all essential amino acids important in human nutrition can be found in soybean protein.

Treatment of the soybean plants with AGROSTEMIN® results in better expansion of root system and the larger number of bacteria nodules on them. Root absorption capacity is increased and therefore the process of formation of above ground portion of the plant is also faster. All until formation of pods, soybean plants have bigger leaf surface with clear green colour, and generally plant habitus is stronger. More intensive development of the plant above the ground and greener colour of leaves suggest more intensive photosynthesis within plants and the results achieved show that there is increase in chlorophyll a and chlorophyll b contents, as well as their total content (a + b) in soybeans plants (23)(11). Also, the positive results of soybean plant transpiration under the influence of AGROSTEMIN® suggest more intensive photosynthesis and photophosphorylation (10)(24). Because of all this, the ultimate effect is in increased biomass (greater number of pods and beans within pods) and better quality primarily in terms of content of raw oils and raw proteins in soybean grains.

The increase of soybean grains yield was 300–500 kg/ha more than in plants not treated with AGROSTEMIN®, or in relative amount of 8–15% more. The increase of raw oil and protein contents in soybeans grain was about 400–700 kg/ha more in comparison with plants not treated with AGROSTEMIN® (25)(26).

Effects of AGROSTEMIN® application on sunflower

Fundamental research have shown that in sunflower *(Helianthus annus sp.)* whose seeds were treated with AGROSTEMIN® the plants had more developed leaf mesophyll, and accordingly thicker leaves, whereas the number of layers of palisade cells was also increased. Palisade cells were more filled with chloroplast, which suggests the increased synthetic potential of

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treated plants (27). Increased content of chlorophyll a and chlorophyll b, as well as their total content (a + b) in plants (23)(10) was also established, as well as increased intensity of photo-phosphorylation and ATP synthesis (24)(10).

In the transpiration process AGROSTEMIN® has positive role in increasing the intensity of respiration in plants, as well as significance in substance exchange in plant–soil system (24).

The results of photosynthesis intensity increase result subsequently in the increase of yield and biomass (larger diameter of capitulum (flower head) and the number of seeds in the flower head), as well as higher quality seeds (primarily higher percentage of oil in the seeds) (25)(26). Thus the yield of sunflower seeds whose plants were treated with AGROSTEMIN® was about 300–500 kg/ha more, and oil percentage was 158.3 l/ha higher in comparison with the yield of seeds and oil percentage respectively of sunflower plants not treated with AGROSTEMIN®.

From the results obtained it has been observed that in treated variants of sunflower plants the interval between germination and buttonization was 2–3 days shorter in comparison with un-treated plants (control), which also made conditions for earlier flowering. In this way the interval between flowering and physiological maturity has been prolonged in sunflower plants treated with AGROSTEMIN® (3 days). It has also been noticed that sunflower plants treated with AGROSTEMIN® show higher resistance to diseases, particularly to *Phomopsis* influence.

Effects of AGROSTEMIN® application on sugar beet

Application of AGROSTEMIN® is one of the agrotechnical measures within the appropriate technology of sugar beet growing.

Based on many years of research of application on sugar beet, in the sprouting stage the differences were noticed between treated and control groups which were even 20% in favour of the former. Better germination energy made conditions for the sprouting stage of treated plants to be 2 days shorter than in control group.

Under the influence of AGROSTEMIN® faster development of root is stimulated and more active interaction between plant and soil is established.

Longer root and better absorption capacity make conditions for faster development of the plant portion above the ground. Plants are more luscious with clearer green colour of leaves, which suggests more intensive photosynthesis and assimilation of organic substances both in the root and in the above ground portion of the plant.

The differences were noticed in the course of vegetation between treated and untreated parts of the plants, which manifested particularly during tropic heat waves, since untreated beets' foliage dried out and in treated beets the foliage remained green during the entire period of vegetation

Effects of AGROSTEMIN® on biological quality of harvest reflect in:

- increased digestion
- increased yield of polarization sugar
- increase maturity quotient value
- increased pressed juice quotient value

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– increased K content in pressed juice
– reduced content of α amino nitrogen in pressed juice.

The most significant fact for farmers is that by using AGROSTEMIN® they can achieve increase in both yield and digestion. Thus the increase of yield in sugar beet treated with AGROSTEMIN® was 5–9 t/ha more in comparison with untreated sugar beet, and digestion was 1–2% higher.

Effects of AGROSTEMIN® application on vegetables

Using the results of fundamental research achieved, the application of AGROSTEMIN® leads to final effect – increase in yield and improvement of vegetable quality in a series of parameters.

The basic effects of AGROSTEMIN® as an ecological preparation for plant nutrition on vegetables reflect in increased yield particularly in increased biomass, which is especially important for vegetables whose main parts of the plant are used for nutrition. This increase in yield depends on a series of parameters, primarily ecological factors and is 20.6% on an average, and it can be seen from Table 1 (29)(30)(31).

Table 1 – Average increase of yield in vegetables under the influence of AGROSTEMIN® depending on the kind of vegetable

A kind of vegetable	Yield (kg/ha)		Difference (kg/ha)	Relative increase (%)
	On treated part	On untreated part		
Potato	26,439.8	20,568.2	5,871.6	28.5
Beans	616.9	495.0	121.9	24.6
Tomato	58,356.0	47,129.0	11,227.0	23.8
Aubergine	93,236.7	74,798.7	18,438.0	24.7
Pepper	33,997.0	30,729.0	3,268.0	10.6
Cucumber	80,331.0	66,268.8	14,062.2	21.2
Cabbage	35,490.0	28,875.0	6,615.0	22.9
Chinese cabbage	62,253.0	57,288.0	4,965.0	8.6

CONCLUSION: Relatively average increase of vegetable yield under the influence of AGROSTEMIN® is 20.6%.

In addition to this, the quality of vegetables is significantly improved, which reflects in higher content of dry matter (for 1–2%), as well as higher sugar content (32). It is not to be disregarded in vegetables that it ripens earlier (7–10 days) and that the size of fruits is more well–balanced, as well as better health condition of plants and fruits. Optimum concentrations and stages of application for various kinds of vegetables are given in the Instructions which come with every package of AGROSTEMIN®.

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Manners of AGROSTEMIN® application

Application of AGROSTEMIN® in the existing technology of cultivating crops does not require any additional financial investments. It is simple for use and dosing, without any special requirements, it is compatible with common agrotechnical measures in terms of manner and time of treatment.

It can be added either to seeds or plants, depending on the available mechanization, type of cultivated crop and the stage of its development at the moment of application (there are instructions).

Based on the research so far, it is possible to use AGROSTEMIN® with both fungicides and herbicides.

Toxicological analyses of AGROSTEMIN® preparation show, which is very important from the aspect of human health and environmental protection, that doses applied on plants are not harmful or that there are harmful residues in human organism, animals and plants (33). There is no waiting period – certificated for organic farming.

It is kept in dry and dark place, shelf life is 10 years.

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