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Innovative bio-products for agriculture

Abstract: The paper reports state of the art research in the field of novel bio-based products for agriculture. Biopesticides, biostimulants and biofertilizers were shown as a potential alternative or additional products to the currently used agrochemicals. The present situation on the market of agrochemicals was discussed. Difficulties related to products registration were taken into account. Research on supercritical algal extracts as a biostimulant of plant growth and health supporting feed additive for laying hens was presented, as well as the perspectives of the use of biomass as the carrier of fertilizer nutrients. The method of production of new fertilizer components with micronutrients was biosorption. The concept of new phosphorus biofertilizers containing living microflora that solubilizes phosphorus was reported.

Keywords: biopesticide, biostimulant, biofertilizer, algal extracts, biosorption, micronutrient fertilizer

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1 Introduction

In recent years, tasks for agriculture have become challenging and sometimes difficult to be met because of conflicting goals. On the one hand, agriculture should be more efficient because it must be able to feed the growing world population with less arable land available. On the other hand, it should be sustainable, organic and able to produce so-called 'bio' products, which consumers began to buy more frequently despite their inferior visual quality. Legislators also pay special attention to the side effects associated with the use of conventional, synthetic agrochemicals: a hazard for consumers(residues), the environment (biodegradability issue, the threat to bees). Perhaps it will support the implementation of new bio-products to the market, some of which may be safe substitutes, others – complementary to currently used agrochemicals [1,2].

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2 Agrochemicals and their role in contemporary agriculture

The scheme of crops production is currently based on two pillars: the use of fertilizers and chemical protection. It would be worthwhile considering the addition of the third pillar - bio-products. Agriculture has recently become a technocratic process. Field crops are intensively fertilized with mineral fertilizers together with the full chemical plant protection. This can lead to soil degradation [3]. Degraded soils are no table to sustain the health of plants. This will result in the opposite effect than is expected: a reduction in production yield. It should be kept in mind that soil is a specific ecosystem in which a balance should be maintained between mineral, organic and biological matter, with the latter being defined as micro- (bacteria, fungi) and macro-organisms (e.g. earthworms, plants).

Without the use of fertilizers, crop production would not be possible. It is necessary to supplement the constituents that were taken from the soil with harvested crops. Currently, the cultivation of plants without the use of full chemical protection is actually impossible. What is more, agriculture became dependent on these preparations. The problem of pest resistance to synthetic agrochemicals has appeared. This is an issue analogous to that which has recently been observed in human therapy: drug resistance of pathogenic microorganisms to antibiotics. This generates a need for the development of new products for agriculture [2,4].

3 Definition and importance of agri cultural bio-products

Bio-products are products of natural origin, and therefore fully biodegradable and non-toxic either to plants and their consumers. As a result, there is no problem of toxicity or ecotoxicity, harmful residues, fate and behavior in the environment. These formulations are safe for the operator of the dispensing agent. Bio-products are derived from microorganisms or macroorganisms, for example by extraction or homogenation. They may also include semiochemical compounds, which are substances used for intra- or inter-species communication, for instance semiochemical biopesticide for cotton pest *Helicoverpa spp.* management [5].

Bio-products fall into three categories: biopesticides, biostimulants and biofertilizers. They differ in purpose of use and mechanism of action. Their role is crop protection, enhancement and nutrition, respectively.

3.1 Biopesticides

Biopesticides are an alternative to conventional pesticides. This group includes, among others, biofungicides, bioherbicides and bioinsecticides. These preparations are effective in small doses, act very precisely, but very narrowly. For example, a bio-insecticide which is a pheromone, being intrusive in hampering the propagation of a particular species of insect-pest is only effective for a given pest species. It will not work against other pests. Synthetic chemicals have a much broader spectrum of action, although we should bear in mind that these are toxic agents – xenobiotics [6-7]. An example is controlling powdery mildew on cucurbit rootstock seedlings by biofungicides (a combination of sulfur, fish oil and sesame oil) [6].

3.2 Biostimulants

While biopesticides protect against biotic stress (i.e., attack by pests), biostimulants protect the plant against abiotic stress (i.e., frost, drought, salinity). The effectiveness of these agronomic products can be assessed by, e.g. rootmass measurement, the intensity of photosynthesis and the extent of the harvested quantity on experimental plots in relation to the control ones and those on which the reference product was used. The effect of biostimulants is very clear when abiotic stress conditions occur. If stress is not present, the differences may not be observed. Biostimulants are thus a means of protection for the plant in the case of abiotic stress [8-11].

3.3 Biofertilizers

Biofertilizers can be described as living fertilizers. The application of a bio-fertilizer does not provide a fertilizer nutrient itself, but instead a method of acquiring this nutrient. Mycorrhiza preparations may serve as an example here. By adding microorganisms fixing atmospheric nitrogen (fungi or bacteria, symbiotic or non-symbiotic – the latter are much easier in manufacture, because they can be propagated in the bioreactor) to the root zone a fertilizer component – nitrogen is provided to plants without the *de facto* application of fertilizer nitrogen itself. Another example is phosforus biofertilizer – phosphate solubilizing bacteria in soil that solubilize phosphorus soil deposits which are not available to plants [12].

4 Bio-products – prospects and problems: registration and implementation on the market

There are some bio-products present on the market. The oldest is seed biofungicide based on *Bacillussubtilis*, effective against fungal diseases caused by the fungi of the genus *Fusarium*, *Rhizoctonia*, *Alternaria*and *Aspergillus*. Although bio-based products offer new opportunities, and their application is completely safe and they have been investigated for more than 50 years, only 0.1% of developed formulations have been put on the market. The reason for this is complex and is associated with the current situation on the agrochemicals market, registration procedures and different working mechanisms than those of conventional agrochemicals. In bio-products there is no particular active substance with identifiable properties, as in chemical synthetic products. In bio-products there is a vast array of compounds that usually act on the basis of synergy. But actually, the exact mechanism of bio-products action still remains unknown.

The time between the discovery of the active ingredient and putting it on the market is estimated at 10 years. The costs associated with product development and registration research are counted in the tensor even hundreds of millions of euros. The cost of registration alone is 1.5 million euro. The idea of restrictive registration procedures was the best possible understanding of the substance characteristics before placing it on the market. However, the effect that has been achieved is quite different. On the agrochemicals market, there is a relatively small number of biologically active substances. Most of these substances were developed over 30 years ago. There is a lack of innovative products. 90% of the market belongs to7agrochemicalcompanies that in fact are not bio-products producers.

This situation on the agrochemical market makes it difficult for bio-product manufacturers which are average business enterprises, to enter this market [4]. For this reason, producers of bio-products began to organize themselves by forming consortia. The European Biostimulant Industry Council (EBIC) was established, whose objective is to develop the legislation for the registration of bio-products, relevant to their specificity. In the US, this problem has already been partially solved and the period of time between the discovery of the substance to the moment of its registration was shortened from 10 to 3 years. At the same time the costs associated with product development were significantly reduced. The EU countries still abide by EU agrochemical regulation (1107/2009). Bio-products (biopesticides, biostimulants) are subject to registration in accordance with the mentioned Regulation. This means that a so-called registration dossier should be submitted to the relevant European institutions EU ECHA or EFSA. The dossier covers a description of the results of research on physicochemical properties (in the case of bio-products – including also biological ones. The documentation concerning toxicology, ecotoxicology, fate and behavior in the environment and residues should be also submitted. However, study of these bio-products characteristics is pointless, because they do not pose a risk in this regard. The application should also include reports on the agronomic effectiveness. The registration dossier presents a characterization of a validated analytical methodology for the determination of the active substance in the product, the plants (the residue) and the environment (water, soil and air) [13].

In the case of bio-products, preparation of such documentation is not an easy task because the separation for the purpose of identification of the active substance is difficult and often even impossible. Bio-products as materials of natural origin (e.g. plant extracts) contain a wide variety of different biologically active compounds (sometimes even morethan100) at low concentrations. It is very difficult to identify a particular compound that *de facto* can be considered as the active ingredient. Such a product can be fractionated into single compounds and agronomic effectiveness of each compound can be assessed. Probably it would be found that none of the present compounds is effective acting separately. The substances operate on the principle of synergy if they are all present in the natural product. Also, such studies are both very difficult and expensive. Inability to identify the specific active ingredient generates further difficulties, e.g.: development of analytical methods, determination of the product stability and shelf-life. Another problem is the reproducibility of the composition. This is an issue associated with almost all natural products. The composition of e.g. plant extracts will be different depending on the conditions of the vegetation season (rainfall, sunshine, soil richness, geographic location).

Another challenge is to prepare the formulation. This is important, because the developed product should not be harmful to the recipient; the preparation of the working solution should be facilitated: the product should not separate into distinctive phases, nor precipitate, so as not to cause clogging of the nozzles of the applicator device, nor cause an uneven distribution of the product. The preparation should not also lose its effect. It is important to note that it contains fragile biologically active compounds that are often hydrophobic or water-immiscible. By adding the appropriate co-formulants, good miscibility with water, product stability and activity should be ensured.

5 Bio-products development – examples

Our own research includes the development of different bio-products for agriculture, including supercritical extracts from algae. Properties of plant growth biostimulants and biopesticides (biofungicides) are researched. Also animal-health supplements are being developed. The trial on 180 laying hens has been initiated in the Vivarium of the Wroclaw University of Environmental and Life Sciences. For 150 days, formulations of the alga extracts will be added to drinking water for animals. The impact on the health of hens, egg production and egg quality (eggshell-breaking strength and thickness, biofortification of egg content of polyunsaturated fatty acids, carotenoids to obtain designer eggs, will be investigated) [14-16].

Research on algae extracts-based fertilizers produced by physical, chemical and biological means has also been carried out [17].

Fertilizers with trace elements based on bio-components are produced by biosorption. Biological carriers of fertilizer micronutrients are the supercritical post-extraction residues of berry seeds. Poland is the main global manufacturer and also a leader in fruit processing. In the process of manufacturing, berry seeds as a by-product that requires utilization are formed. The seeds can be used in the production of essential oils by the supercritical extraction method, useful for the food, cosmetic and pharmaceutical industry. After this process, residues requiring disposal are formed. It turns out that they have very good sorption properties: they bind cations from a solution in the biosorption process, which was found useful in the production of micronutrient fertilizers. Since it is an ion-exchange mechanism, the resulting product has characteristics of a fertilizer with a controlled, sustained release of nutrients. New components provide a micronutrient fertilizer with a high bioavailability and a low leaching rate into groundwater. This has been proven in the *in vitro, in vivo* laboratory (Petri dish tests) and *in vivo* field experiments. In the field studies on corn, a greater efficiency of crop production was obtained. The biofortification of corn grain with micronutrients was an additional effect. This gives rise to the development of a method of obtaining seeds of maize with an increased density of trace elements, which could be useful in the prevention of the microelement deficit in a human organism, and as an alternative to the mineral supplements [18-19].

Among some other bio-products for agriculture are phosphorus bio-fertilizers with phosphorus solubilizing living microflora. Microorganisms solubilize phosphorus deposits present in the soil that are not available to the plants. The microflora of *Bacillus megaterium* and *Acidithiobacillus Ferroxidans* is propagated on renewable raw phosphate materials (bones, fish bones, ashes of phosphorus sludge), which turned out to be a good source of phosphorus in the growth medium as compared to non-renewable raw materials (phosphates). Bio-fertilizers containing living microflora are applied to the soil and at the same time provide certain doses of phosphorus, but smaller than conventional fertilizers. Living microflora solubilize soil phosphorus not available to plants and causes dissolution into the soil solution, from which plants can take up the fertilizer nutrients by the root system [20].

6 Examples of innovative bio-products for agriculture

There are different classes of plant growth biostimulants: microbial inoculants, humic acids, fulvic acids, protein hydrolysates, amino acids, and seaweed extracts [21]. Saa et al. [22] investigated foliar application of microbial and plant based biostimulants and found that potassium uptake in almond (Prunusdulcis) and plant growth increased. The biostimulants were extracts from *Ascophyllum nodosum* and microbial fermentation. A significant beneficial effect of the biostimulants on plant growth was observed (shoot length and biomass, total shoot leaf area under potassium deficiency), however the mechanism of action has not been identified.

Biostimulants from seaweed and black peat were applied on winter oilseed rape to test response in the mineral content and gene expression [23]. The preparations stimulated root growth and macronutrient uptake (N, S, K, and P) and also chloroplast division. Enhanced plant concentrations and root-to-shoot translocation of Mg, Mn, Na, Cu, Fe and Zn were observed. A Cu transporter that is responsible for translocation of Fe and Zn was expressed. This shows the possibility of plants biofortification by using biostimulants.

Tian et al. [24] supplemented macronutrients and microbial fermentation products to improve the uptake and transport of foliarly applied zinc in sunflower (*Helianthus annuus*) by the micro X-ray florescence method when microbially derived organic biostimulant “GroZyme” was applied. Mobility of Zn from different preparations and phloem loading as the result of using an organic biostimulant were determined. Zinc was visualized within the vascular system of the leaf petiol and enrichment of Zn in the vascular tissues was confirmed. The authors showed the mobility of Zn and its absorption by the sunflower leaf.

Rodríguez-Morgado et al. [25] obtained edaphic biostimulants and biofertilizers from sewage sludge and studied their effects on soil biological properties. Sewage sludge was processed by anaerobic fermentation/aerobic processes with/without maturation and was autoclaved/not autoclaved. Soil enzymatic activities were stimulated significantly and were found to be related with the presence of lower molecular weight proteins. Temporal variations in the soil bacterial community structure (16S rDNA-DGGE profiles) were confirmed.

Przybysz et al. [26] tested the biological mode of action of biostimulant based on nitrophenolates (“Atonik”). The influence on plant yield and yield parameters (crops), biochemistry (crops and model plant), morphology and physiology were shown and biostimulatory effect was confirmed.

Colla et al. [27] studied biostimulant action of protein hydrolysate containing amino acids and small peptides derived from plant by enzymatic hydrolysis. Hormone-like activity, nitrogen uptake, and growth stimulation were investigated in terms of biostimulant action on corn, tomato and pea. Coleoptile elongation rate increased as the result of applied biostimulants and the auxin-like effect was observed. Resulting hormone-like activity, enhanced nitrogen uptake and consequently enhanced crop performances were observed. Another example of protein hydrolysate used to enhance the growth of plants was biostimulant produced from chicken feathers and sewage sludge containing peptides and amino acids [28]. Its effect on enzymatic activities and the structure of the soil microbial community (phospholipid fatty acids) was evaluated. The authors found that biostimulant applied on chlorpyrifos-polluted soils decreased the inhibition of the soil enzymatic activities and biodiversity and had soil remediating effect.

The properties of biostimulant derived from plant on metabolic profiling and crop performance of lettuce grown under saline conditions (abiotic stress) was investigated [29]. Growth, root morphology, leaf mineral composition, SPAD index, chlorophyll fluorescence, and metabolic profiling were improved, in particular when biostimulant was applied to boths shoots and roots.

There are many examples found in the recent scientific literature that support the agricultural efficiency of these products by enhanced root growth, uptake of nutrients and tolerance to stress. Although these examples show that undoubtedly, bio-preparations enhance the growth of plants, the mechanism still remains unknown. Some studies concern commercially available bio-products, others describe the new preparation.

7 Conclusions

Bio-products are very prospective products in modern agriculture. However, their presence on the market will be strictly conditioned by the removal of legislative obstacles. The registration directive should therefore include the specificity of bio-products, in particular, difficulties with the identification of a particular active ingredient. When these formal obstacles are overcome, it is expected that modern products, safe for plants, the environment and, most importantly, for human-consumers, will appear on the market.

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