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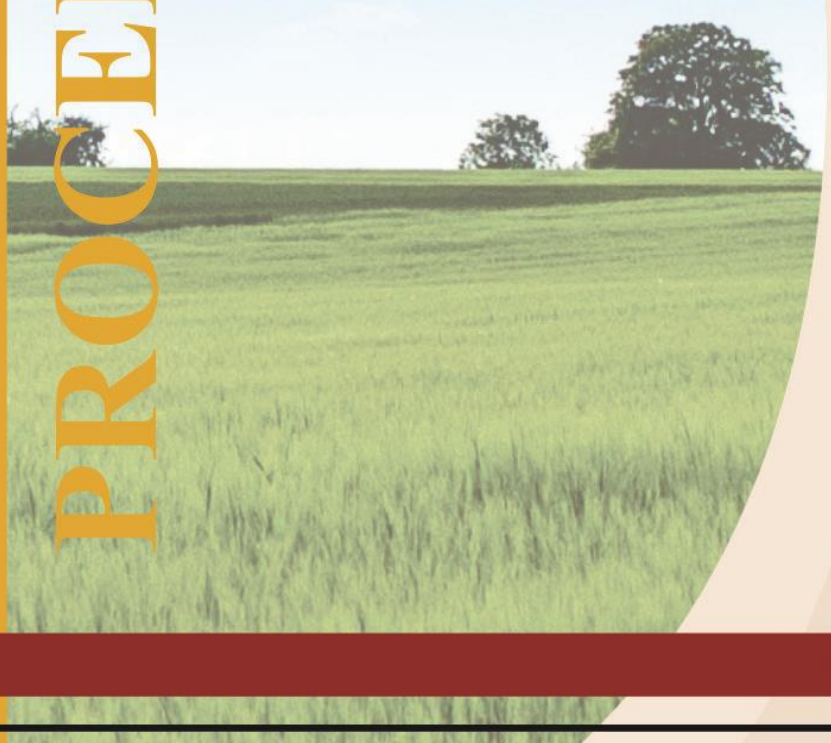

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DIFFERENT ASPECTS OF NON-STANDARD FOLIAR FERTILIZERS BASED ON AMINO ACIDS, PHYTOHORMONES AND PLANT EXTRACTS

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Abstract

We studied different aspects of application of foliar non-standard fertilizers based on amino acids, phytohormones and plant extracts. The trials were carried out at the level of the seedlings, individual plants grown in semi-controled conditions and plants grown in the field. Various energetic and thermodynamic parameters were analyzed, then the chemical composition (mineral elements, different sugars, secondary metabolites, etc.), as well as parameters of plant growth and their yield, in order to better assess the impact of these fertilizers on crops. We found that on maize seedlings it works by changing the content of various elements, then the polyphenol profiles, as well as thermodynamic parameters, where this effect does not only depend on the dosage of the fertilizers, but also on the corn genotype. We also found that the fertilizers affect the energetic and thermodynamic parameters of individual maize plants, as well as the parameters of plant growth. The most significant and most diverse results were obtained by analyzing the yield and components of the yield of many different crops (crop, fruit, vegetable), as well as their chemical composition (mineral elements, different sugars, secondary metabolites, etc.) in terms of improving nutritive quality. It was noticed that these fertilizers greatly affect the content of microelements, starch and crude proteins in maize and barley, sugar and polyphenol content in various fruit trees, as well as in soybeans, in which we note that in certain agroecological situations these fertilizers have led to spectacular magnification yields of different crops, but there were also situations when they did not have any positive effect on crop yields. Overall, the early treatment of cultivated plants with non-standard fertilizers greatly affects the vigor of seedlings of these plants, which is of great importance for crop yield. These fertilizers also significantly affect the quality of crop yield in terms of improved chemical composition of edible parts of these plants.

Keywords: *Amino acids, brassinosteroid (BRs) phytohormones, plant extracts, plant protection, resistance of plants to stress, biofortification*

Introduction

Unlike "classical" fertilizers, which are used as agro-technical measures to supply plants with certain elements, their treatment of non-standard fertilizers is primarily based on the intensification of plant metabolism, either due to their treatment with specific metabolites (eg, amino acids), either phytotohormones, or plants extracts, containing all these substances. This acceleration of plant metabolism occurs due to increased synthesis of protective substances, more intensive adoption of some important nutrients or due to the presence of additional signal substances outside. This path affects not only the yield of crops in quantitative terms, but, more importantly, their reaction to specific agroecological situations of abiotic, biotic and xenobiotic stress, as well as the change in some qualitative yield parameters in terms of increasing the nutritional value of economically usable parts of plants. Thus, more effects are

achieved, which do not necessarily have to be related only to crop mineral nutrition, but also to their increased resistance to stressful situations, and also to the possibility of bio-conditioning of crops in a kind of organic food production. Unlike our previous release, which relates only to corn and fertilizer based on brasinosteroid phytohormones (Waisi *et al*., 2015), we give a broader overview of crops and types of applied non-standard fertilizers.

Materials and Methods

Plant growth and yield parameters are described in our previous work (Waisi *et al.*, 2015). The thermodynamic parameters are defined in usual manner (Sun, 2002), and their calculus is described in our previous works (Waisi *et al*., 2017a, 2018). Total polyphenols, starch, sugars, crude proteins, oils are described as in our previous work (Waisi et al., 2015), where a more detailed description of the methods of quantification of total polyphenols, proteins and polyphenolic fractions and antioxidative plant tissue capacity is given as in another our work (Đurović et al., 2019), while the description of the quantification of certain sugars was given in an PhD thesis (Waisi, 2016). The quantification of the elements was performed using the AAS method (Waisi *et al*., 2015, with more detailed in Waisi *et al*., 2017b).

Results and discussions

In our announcement (Nikolić and Waisi, 2012), we examined the results from micro-trials, wich where set up in 2011 in two apple orchards located at northern part of Serbia. Plots were treated with combinations of half of the usual dose of mancozeb and tebuconazole fungicides as a controla, and also same treatments combined with brassinosteroid (BRs) based preparation and also with non-standard fertilizers based on amino acids and plant extracts. First, we evaluated the usual parameter of yield of fruits, and the apples were sampled for determination of content of reducing sugars in extracts of fruit pulp. Also we assesed efficacy of these procedures to plant protection od apple leaves and fruits from notorious phytopatogenic fungus *Venturia inaequalis* (Stevanović *et al*., 2012). In Obrenovac trial evaluated yield/ ha of BRs treated apples is same as in control plots, with comparable pomological and fruit quality parameters of apple. In Šid trial evaluated yield/ ha of BRs treated apples for almost a quarter more then apple yield from control plots (treated by half and full doses of fungicides) and other treatments, also with comparable pomological and fruit quality parameters of apple fruits (data not shown). From plant protection view, our procedures is also satisfy with 78,71% and 77,69% protection efficacy of BRs+half fungicide doses treatment in leaves and fruits (against 84,17% and 87,90% efficacy from full fungicide doses treatment) at Obrenovac, which is satisfactory result. In *Šid locality* we got similar results, whith also satisfactory results (not shown). Our results are very similar to findings by other researchers (Khripach *et al*., 2000).

Also, we examined influence of non-standard fertilizers on yield and yield components in soybean and barley. During 2012 sezone 3 soybean genotypes (ZP-015, "Nena", and "Laura" with low content of Kunitz-trypsin inhibitor protein) was treated with a non-standard fertilizers, as type of biofortification. By this approach we found that it is in lesser extent affected by alterations in P_{phy} (content of phytic phosphorus), as important factor which restrain availability of mineral nutrients. Only at the Zn level, this dependence is significant, where lowering in P_{phy} increase parallel Zn concentration in grains. Moreover, the influence of β-carotene is significant for availability of mineral nutrients, but more important is that its increase is linked with parallel Fe increase, mainly in grains with higher weight, as part of better yielding potential. It is significant to underline that ratio between P_{phy}, β-carotene and mineral nutrients could be altered in some degree by application of foliar fertilizers with potentially higher availability of minerals, but it also depends on soybean variety. 24-epibrassinolide (24-EBL) based preparation and the plant extract ("Zircon") were efficient for

decrease of mentioned ratio for ZP-015 and "Nena" grains, as well as some plant extracts ("Zlatno inje" and "Zircon") were efficient for "Laura". Also, in soybean is very significant correlation between 1,000 grain weight (as important yield component) and grain content of β-carotene and Zn (Dragičević *et al*., 2016b). At a beginning of seasons 2013 and 2014 we sown hull-less barley (*Hordeum vulgare* L. var. *nudum*; cv. "Apolon"), after that in the spring of the years, we treated the crop with BRs based preparation, and with other non-standard fertilizers (based mainly on plant extracts and other bioregulators). After harvesting in the summer we assessed yield (at 14% grain moisture content; kg ha^{-1}) and determined by standard methods different chemical ingredients from barley grains. Obtained results (Dragičević *et al*., 2016a) indicate that year affects barley grain yield and its chemical composition, with the highest impact obtained for Si under harsh climate conditions. Applied treatments was the most effective for grain yield and increase of grain quality mainly across reduction of P_{phy}/β-carotene ratio and increase of GSH content, thus increasing potential bioavailability of the mineral elements. What is more, stress present in high precipitation amount (in 2014) could be mitigated by application of *an* fertilizers by increasing potential bioavailability of P, Mg, Ca and Fe. Generally, BRs preparation influenced content of P_i, Zn and Fe, and other fertilizers mainly affected potential availability of some other mineral elements (Ca, Mn, Si and GSH).

From previous field trials carried out on one fruit and two field crops we indicated that *beetwen* other non-standard fertilizers, preparation based on BRs affects not so much yield, as to yield quality or chemical composition (Nikolić and Waisi, 2012; Dragičević *et al*., 2016a, 2016b) and protection to the crops in stress conditions (Stevanović *et al*., 2012).

In our paper (Đurović *et al*. 2019) the influence of different non-standard fertilizers on the content of polyphenolic acids and proteins in soybean seed was monitored. It is also followed the antioxidative capacity of soybean seed (as important soybean nutritional feature) by various methods: Total phenol content (TPC; not shown), free radical scavenging activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radikal assay, ferric reducing ability of plasma (FRAP) assay as a reducing power of soybean extract (Figure 1), and the Briggs-Rauscher (BR) reaction method (Figure 2). All examined plant-extract-based products expressed significant changes in the total phenolic contents and antioxidant activities of the soybean flour extracts. An exception was the treatment with "Cropmax", which only caused a decrease in TPC and antioxidant activities determined by the DPPH and FRAP methods. All other treatments showed a positive influence on the TPC, DPPH and FRAP methods, which is in accordance with several studies (Danilčenko *et al*., 2017; Verkleij, 2012) that showed a positive influence of biofertilizers (based on plant extracts) on the yield, growth and antioxidant activities of different plant species. The results of the post-hoc Tukey test showed that all treatments significantly effected a change in TPC as compared to the control sample. A statistically significant difference in antioxidant activity was not found between the samples treated with "Ekofus" and "Vegard", while all treatments in relation to the control sample exhibited statistically significant differences in antioxidant activity as measured by both DPPH and the FRAP methods. Inhibitory effects after the addition of aqueous extracts of soy flour to active Briggs-Rauscher (BR) mixture were reported (Cervellati *et al*., 2000). However, to the best of our knowledge, this is the first time that soybean treated with products based on plant extracts during vegetation were analyzed in the oscillatory Briggs-Rauscher reaction. It is well-known that the BR reaction method provides a "larger antioxidant picture" and can also show a synergistic effect (Milos and Makota, 2012).

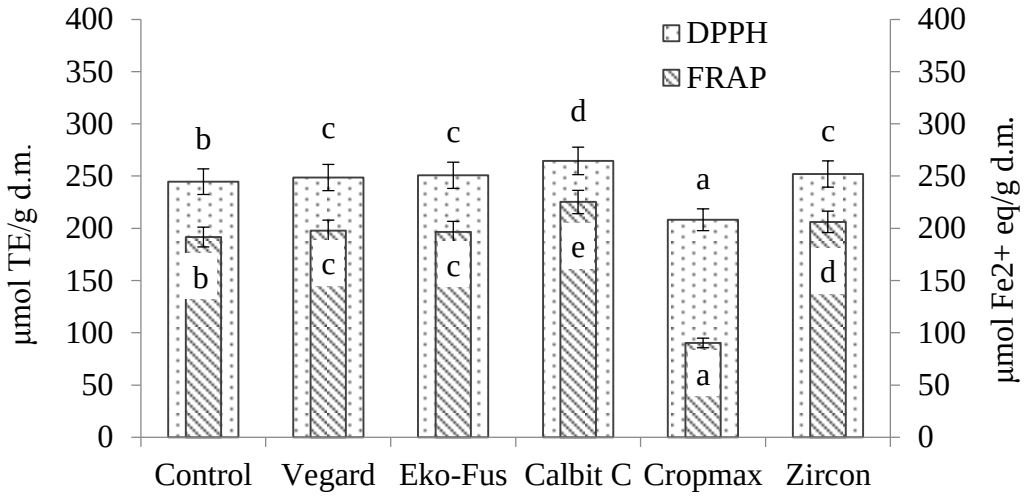

Figure 1. Antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing ability of plasma (FRAP) assay in soybean seeds treated with plant extract products. The values followed by the same letters are not significantly different at the 0.05 level

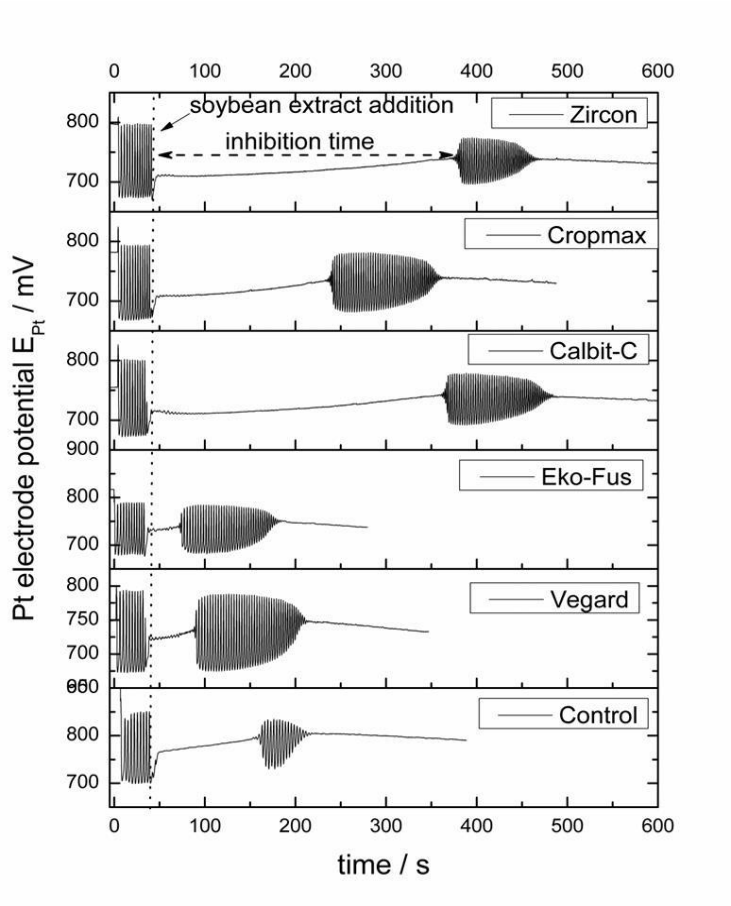

Figure 2. The Briggs-Rauscher oscillograms obtained with particular soybean extract addition (100 µl) after 30 s from oscillatory reaction beginning. The initial concentrations of reactants for BR reaction were $[CH_2(COOH)_2]_0$ = 0.0789 mol/dm^3, $[MnSO_4]_0$ = 0.00752 mol/dm^3, $[HClO_4]_0$ = 0.0300 mol/dm^3, $[KIO_3]_0$ = 0.0752 mol/dm^3 and $[H_2O_2]_0$ = 1.2690 mol/dm^3.

Therefore, the results obtained by the BR reaction method demonstrated the synergistic effect (of phenolic compounds and proteins) in soybean treated with "Cropmax", as well as a more favorable outcome of soybean treatment with "Calbit-C" and "Zircon". This indicated that not only phenolic compounds participated in the inhibition of the oscillatory regime, but also other molecular species (such as proteins and some ions), which influenced the BR reaction, possibly by building and/or stabilizing macromolecular structures in plant cells.

Also we tested (Waisi *et al*., 2017) a brassinosteroid (BRs) phytohormone based fertilizer and their influence on plant growth and microelements accumulation in seedlings of two maize genotypes. It was found that BRs influencing both germination and growth of maize hybrids ZP 704 and ZP 434 at their lower concentrations. Hybrids divergently reacted to exogenous treatment by brassinosteroids. Lower BRs concentrations stimulated seed germination and growth of seedlings, but high concentrations inhibited these processes. Considering that germination percentage, whole plant mass and their *innitial* height as a vigour parameters of plants, seedlings treated with various concentrations of brassinosteroids will probablly have better chance for growth and field establishment. It was found that BRs is affecting redistribution of elements in young plants. Elements could be linked with initial growth and it was assumed that in the case of hybrid ZP 704, poorer emergence of shoots is influenced with lower Zn concentration, since it is known that the Zn and plant growth are correlated. In case of the lowest concentration of brassinosteroid (BRs) phytohormone is obvious blocking distribution of Cu, and these could mean that maize plants could achieve optimum of growth in polluted soils. Low values of ratio of photosynthetic pigments for both hybrids are confirming photosynthetic inactivity. Results are implicating from our findings that maize treated with BRs could be grown at soil polluted with heavy metals due to its ability to remove or block accumulation of toxic elements, especially in shoot.

Conclusions

Overall, the early treatment of cultivated plants with non-standard fertilizers greatly affects the vigor of seedlings of these plants, which is of great importance for crop yield. These fertilizers also significantly affect the quality of crop yield in terms of improved chemical composition of edible parts of these plants.

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