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IMPACT OF THE BIOLOGICAL FERTILIZERS ON CHEMICAL INDEXES AND ENZYME ACTIVITIES OF SOILS AT CUCUMBERS

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ABSTRACT

The present experimental work is set as a vessel experiment in a non-heated greenhouse of department „Plant Production", Technical University – Varna, with purpose to be established the role of three biological products (Ekstrasol, Herbagreen, Life Bat Guano) and one mineral fertilizer (NPK) on chemical and enzyme indexes of soils at growing of greenhouse cucumbers variety Kiara F_1.

It is established by the carried out analyzes, that the biological fertilizers leave the soil well stored with total phosphorus and potassium, as the highest results are reported at usage of Ekstrasol. The highest values of total nitrogen are registered at the variants with mineral fertilizing. The catalase activity is higher at the combined fertilized soils with NPK + Herbagreen, Ekstrasol + Herbagreen and Life Bat Guano + Herbagreen in comparison with the controls and the samples, fertilized only with one product (NPK, Ekstrasol and Life Bat Guano). The bringing in of fertilizers increases the activity of the enzyme cellulase at all fertilized soils in comparison with the controls, as this tendency correlates with the increased content of nutrient elements nitrogen, phosphorus and potassium after the adding of the fertilizer products. The activity and of both enzymes may serve as a sensitive bioindicator at fertilized soils.

KEYWORDS – Catalase, Cellulase, Fertilizer products, Nitrogen, Phosphorus, Potassium

1. INTRODUCTION

During the last years the interest towards the greenhouse production of vegetables increases. It occupies hardly 0,4% from all areas, on which are grown vegetables. Thanks to its high intensity of production, the Bulgarian greenhouse production provides 15-17% from the totally produced vegetables in the country. The share of the greenhouse produced tomatoes is almost 50%, and at the cucumbers the prevailing part of the produced quantities (around 80%) are grown in greenhouse conditions [1]. At this production the risk from pollution of the soil, waters and the production is high. A basic reason is the intensive and unilateral usage of the soil and the closed character of the greenhouse production, which creates conditions for usage of high fertilizer norms and pesticides. This imposes the usage of alternative, environmentally sound decisions, providing favourable and economically effective nutrient regimen, which creates conditions for display of the maximum productive possibilities of the plants.

One of the environmentally sound decisions for nutrition of the plants is the applying of bioproducts, which influence not only on the soil microflora, but also on the enzyme activity.

The lack of researches for the usage of bioproducts at the greenhouse cucumbers motivates the present development, in order to be guaranteed the effect from the application of the bioproducts, for preservation of the environment and its potential for the future. The obtained results shall complement and broaden the former researches and would have an important significance for solving of some practical tasks of the contemporary biological agriculture, connected with the maintenance of the nutrient regimen.

The soil microorganisms are important for the enzyme degradation of complex organic substances and for the release of nutrient substances and microelements from the mineral fraction of the soil. They usually occupy less than 1% from the volume of the soil, but their activity is high, since it not always depends only on their quantity. The number and the activity of the soil microorganisms are dependent on the crops, the type of the soil, cultivation of the soil, the macro- and microclimate of each place [2]. Twenty year researches of Zhang et al. (2015) [3] show that the fertilizing with urea (600 kg N/ha) increases the yields by 31%, and the fertilizing with a combined fertilizer (urea and compost) – by 69%. According to these authors the fertilizing with urea increases the values of the enzymes a-galactosidase and β-galactosidase, but it decreases the activity of α-glucosidase and β-glucosidase, which shows that the fertilizing only with non-organic fertilizers has different impact on the enzymes, which participate in the carbon transformation in the soil. The cellulase also participates in the transformation of the carbon – it catalyzes the hydrolysis of the cellulose, at which initially the cellulose disintegrates to cellobiose, which under the action of ß-glucosidase disintegrates to glucose. As a basic destructor of cellulose in the soil, this enzyme at great degree characterizes the direction and the speed of the mineralization processes in it. Researches of Liu et al. (2010) [4] show that the soil enzyme activities are low in not fertilized and N-fertilized soils, but they increase significantly when organic N is applied synchronously. Gaind and Nain (2012) [5] established that the combined fertilizing with organic and non-organic fertilizers, which contain nitrogen and phosphorus increases the activity of the enzymes dehydrogenase, alkaline phosphatase, cellulase, cellobiase and urease in comparison with soils, which are fertilized only with organic fertilizers.

In the processes of transformation of the substances and energy in the soil an important role play the oxireductases, in particular the enzymes catalase and peroxidase. In the molecule of these enzymes participates iron. The mineralization of the organic compounds, which contain iron is provoked by many microorganisms with heterotrophic type of nutrition – bacteria, fungi and actinomycetes. The catalase activity of the soils is one of the sensitive indicators, which defines their biochemical characteristics. Ştefanic et al. (1984) [6], Ştefanic and Picu (1989) [7] established that the catalase activity is higher, while the activities of the enzymes dehydrogenase, invertase and phosphatase are lower at fertilizing with mineral fertilizers, which contain nitrogen and phosphorus, than in not fertilized control soil. Samuel et al. (2008) [8] proved that the organic fertilizing leads to significantly higher increasing of the activity of the enzymes dehydrogenase and catalase in comparison with the application of non-organic fertilizers. Similar results are reported and by some other researchers [9,10], who, however, have established an increased enzyme activity of the soil at combined application of organic and non-organic fertilizers.

Usually the fertilizing strongly advantages the accumulation of bacterial residua [11,12] and increases microbe biomass of the soils [13,14,15,16]. In long-term experiments the fertilizers may influence not only on the structure of the community and the quantity of the soil microorganisms, but also on their functions [17,18]. The activity of the enzymes, as a function from the activity of the soil microorganisms, is an important indicator for the biological condition of the soils.

The purpose of the present development is to be studied the impact of biological fertilizers on chemical and enzyme indexes of the soil at growing of greenhouse cucumbers variety Kiara F_1, as well as to be established the impact of the bioproducts on the soil fertility.

2. MATERIAL AND METHODS

The experimental work is carried out in year 2015 in a not heated greenhouse of Technical University – Varna with greenhouse cucumbers variety Kiara F_1.

Production of seedlings

The seed were put on 01April 2015 in petri dishes, as after germination in a thermostat they were planted in plant pots with volume 0.5 l, full with turf-perlitic mixture. In period 3-4 real leaf they are transferred in plant pots with volume 12 l. The experiment is set as vessel in 3 repetitions. Before planting of the plants there is carried out an agrochemical analysis of the soil. There is followed the nutrient reserve of the soil with nitrogen (ammonium and nitrate), movable forms of phosphorus, absorbable potassium and pH of the soil solution.

Scheme of the experiment

There are used three biological fertilizers – Ekstrasol, Herbagreen, Life Bat Guano. The experiment is set in seven variants as per three repetitions as per the following scheme:

1. NPK;
2. NPK + Herbagreen;
3. Ekstrasol;
4. Ekstrasol + Herbagreen;
5. Life Bat Guano;
6. Life Bat Guano + Herbagreen;.
7. Control – not fertilized

Ekstrasol is a microbial liquid fertilizer, which contains risospheric bacteria from genus *Bacillus subtilis*, content of dry substance not less than 19 %, organic substances 58-64% from the dry substance, humic acids 50-85% from the organic substance, fulvic and low-molecular organic acids 15-50% from the organic substance, potassium not less than 9 % from the dry substance. Salts of the humic acids 80-90 % from the dry substance, microelements.

Herbagreen is 100% natural product, which is obtained from calcitic microparticles ($CaCO_3$). It acts reinforcing to the plants and to the activating of the primary and secondary metabolism. This advantages for the better steadiness of the plant against the biotic and a biotic factors. It contains CaO 44.10 %, MgO 2.20 %, Fe_2O_3 1.20 %, Al_2O_3 – 0.70 %, SiO_2 9.10 %, SO_4 0.11 %.

Life Bat Guano is a natural fertilizer from bats, with rich composition of macro and microelements, as well as enzymes. The organic substance is 20 %, nitrogen 2 %, pH 4.2-6.3. The enzymes and the containing calcium and magnesium stimulate the soil microorganism and under their impact the nutrient substances from the guano are released gradually. It contributes for the better and easier absorption of the nutrient substances from the plants. The guano improves the quality and the production of the obtained crop.

In the carried out experiment there is used mineral fertilizer NPK as a variant for comparison of the biological products.

The fertilizing norms are recalculated for the separate variants. At the variant with NPK there is brought in as per 2 g, as the quantity is applied before the plants to be pricked out. Ekstrasol is brought in at period beginning of flowering, in dose of 2 ml from the fertilizer. Herbagreen is applied in period 6-7 leaf, as it is prepared as a working solution 40 g in 10 l water. Each one of the plants was sprayed, as the treating is carried out from bellow upwards. Life Bat Guano is applied 3 times at an interval of 15 days, as the plants are also sprayed from bellow upwards, in dose of 5 ml.

The content of nitrate and ammonium nitrogen is specified photometrically with Nitrospectral as a result of extraction with solution of calcium dichloride ($CaCl_2$) (ISO 14255:2002).

The content of movable phosphates and absorbable potassium is specified as per standard ISO 11263:2002, through double lactate method of Egner-Riehm. The method is based on extraction of the movable compounds of phosphorus and potassium with solution of calcium lactate $(CH_3CH.OH.COO)_2Ca$.

The active reaction of soil (pH) is specified in water extract, in compliance with Bulgarian State Standard ISO 10390:2011.

The data are included in a dispersion and correlation analysis, as there is used statistical product SPSS for Windows version 16.

The sampling of the soils for analysis of the enzyme activity is carried out by a sterile instrument, in sterile paper bags. The samples are transported and analyzed latest up to 48 hours, as during the period until analysis, they were stored in a refrigerator at 4-10◦ C.

The cellulase activity of the samples is analyzed through a laboratory experiment [19]. In petri dishes there is sprinkled soil with thickness around 7 mm, as there is maintained 60 % Field Moisture Capacity. Over the soil there are put three 3 bands sterile filter paper with sizes 10/50 mm. The cultivation is in a thermostat at 25 °C. At an interval of 15 days is reported percentage of dissolved area with the assistance of a net-standard. The catalase activity of the soil is specified as per manganese-metric method [19].

The statistical processing of the data from the enzyme indexes includes calculation of average value out of three repetitions for the two enzymes and a standard deviation at the catalase.

3. RESULTS AND DISCUSSION

In table 1 are presented the control agrochemical analyses for the content of total nitrogen, phosphorus and potassium, carried out before setting of the field experiment. It is established that the soil is well stored with total potassium (830,81 mg/kg) and total phosphorus (440,57 mg/kg). There is reported a low value of the summary (total) nitrogen (6,65 mg/kg). The soil reaction is slightly alkaline (pH 7,3) and it is favourable for the development of the plants.

Table 1: Content Of Total Nitrogen, Phosphorus And Potassium Before Setting Of The Experiment

Sample	pH	Total K, mg/kg	Total P, mg/kg	Total N, mg/kg
Soil	7,3	830,81	440,57	6,65

The agrochemical analysis after harvesting evidences for one and the same tendency regarding the concentrations of three of the total forms of the studied macroelements in the soil at cucumbers. With highest content is the total potassium, followed by the total phosphorus and with lowest values in all variants is the total nitrogen (fig. 1).

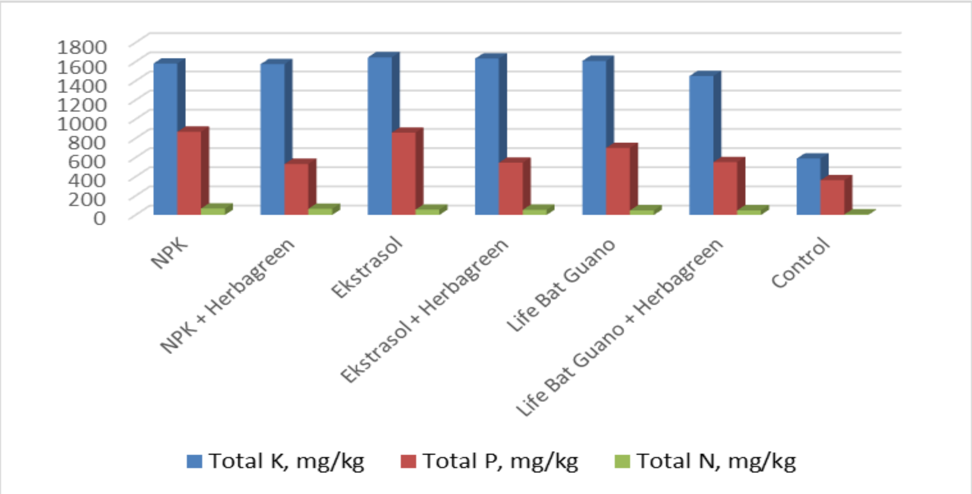

Figure 1: Content Of The Total Nitrogen, Phosphorus And Potassium In The Soil After Harvesting

From the soil analysis is established that highest values of the total potassium are reported due to usage of the biological fertilizers Ekstrasol (1646,19 mg/kg) and Ekstrasol + Herbagreen (1632,54 mg/kg), which statistically proven excel the rest variants. With a close value is the sample with brought in Life Bat Guano (1608,1 mg/kg). In comparison with them, the quantity of total potassium at the variants fertilized with mineral fertilizer NPK and NPK + Herbagreen is with around 5% less than the biological variants. The possible reason for this is the good combination of the used biological preparations, which on their behalf stimulate the soil microflora, they render impact on the enzyme activity and influence positively on the slowly releasing of the nutrient substances. The lowest reported value of total potassium is at the variant with the combined fertilizing Life Bat Guano + Herbagreen (1450,67 mg/kg).

Regarding the obtained results for the quantity of the total phosphorus, there clearly are outlined the variants with fertilizer products NPK (868,51 mg/kg) and Ekstrasol (859,11 mg/kg), which statistically reliable exceed the rest variants. The lowest content of the nutrient element is established at the combined fertilizing NPK + Herbagreen (532,13 mg/kg).

Regarding the total nitrogen, after harvesting of the production the soil is with poor nutrient reserve. With highest statistically proven values are the variants with mineral fertilizing NPK (67,13 mg/kg) and NPK + Herbagreen (64,48 mg/kg). From the biological fertilizers highest results are reported at Ekstrasol (55,68 mg/kg) and Ekstrasol + Herbagreen (53,72 mg/kg). With lowest values are the variants with brought in Life Bat Guano (47,79 mg/kg) and Life Bat Guano + Herbagreen (48,56 mg/kg).

On figure 2 is presented a chart on basis the obtained results from the agrochemical analyses before setting of the experiment and after harvesting.

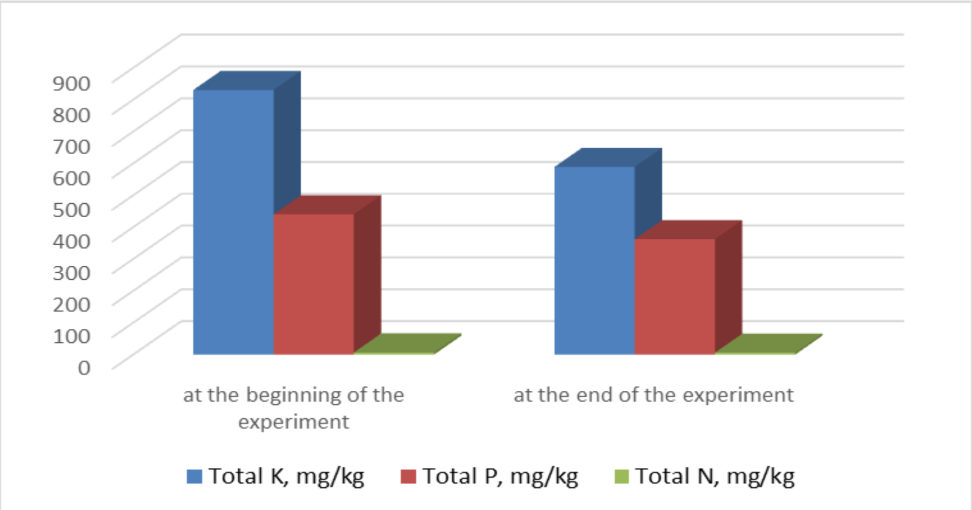

Figure 2: Values Of Total Nitrogen, Phosphorus And Potassium In The Soil At The Beginning And At The End Of The Experiment

There is reported decrease of the quantities of the studied macroelements at the end of the experiment in comparison with the stage of its setting. The percentage of reduction is biggest at the total potassium, followed by the total phosphorus. There can be reported, that there is almost no change in the concentration of the total nitrogen in the control samples at the beginning and at the end of the experiment. A reason for this are the botanical and biochemical characteristics of the cucumbers. It is known that at their initial growth they absorb from the soil in same proportions nitrogen and potassium. During the fruit-bearing the cucumber assimilates 1,5 times more potassium, than nitrogen. In the first period the epigeous part of the plants develops slowly, but a root system is intensely formed. At the same time are set the germs of the generative organs. A leading role in the mineral nutrition in this period is provided of the phosphorus fertilizers [20].

The catalase activity of the soil microorganisms is lowest at the variant fertilized with NPK, as well as at the soil, taken before beginning of the experiment (table 2).

At the combined fertilizing with NPK and Herbagreen the activity of the enzyme increases 1,8 times in comparison with the fertilizing only with NPK and shows highest activity towards the rest variants. It is established that the catalase is higher and at the rest variants with combination of the preparation Herbagreen (Ekstrasol + Herbagreen and Life Bat Guano + Herbagreen). The activity of the catalase is lower with 1,5 times at the variants fertilized with Ekstrasol and Life Bat Guano in comparison with this with highest activity of the enzyme (NPK + Herbagreen). With a close value of the enzyme to Ekstrasol and Life Bat Guano is the not fertilized control sample (1,38 ml O_2/30 min). Obviously not only the better nutrient reserve with the nutrient elements nitrogen, phosphorus and potassium at the fertilized soils in comparison with the controls, but also the combining of more than one preparation leads to increase of the activity of the catalase. According to Uzun and Uyanöz (2011) [21] the activity of this enzyme in the soils depends and on the content of clay, moisture of the soil, the soil depth, the temperature of the soil, the organic matter, pH, the nutrient substances, the microbe content and activity. Also according to these authors, the regimen of fertilizing and alternation of the crops influence on the soil catalase. Although the enzymes are predominantly of microbe origin, they also may descend from plants and animals. These enzymes are continuously synthesized, they can accumulate, inactivate and/or decompose in the soil, and have great significance for the agriculture, because of their role in the decomposition of the nutrient substances [22,23,24,25]. A strong impact on some enzymes in the soil renders the bringing in of mineral fertilizers. Myanusheva (2005) [26] studies the ureasic, proteas, catalase and cellulase activity of soils at bringing in of different quantities of carbamide. It is established that only the catalase activity is not influenced by the brought in different quantities of this preparation. This result supports the obtained lowest result at our research of the enzyme at the sample, fertilized with NPK. And at both fertilizer preparations the content of nitrogen is highest.

In contrast to the catalase, the cellulase activity is influenced at high degree at all variants of fertilizing (fig. 3).

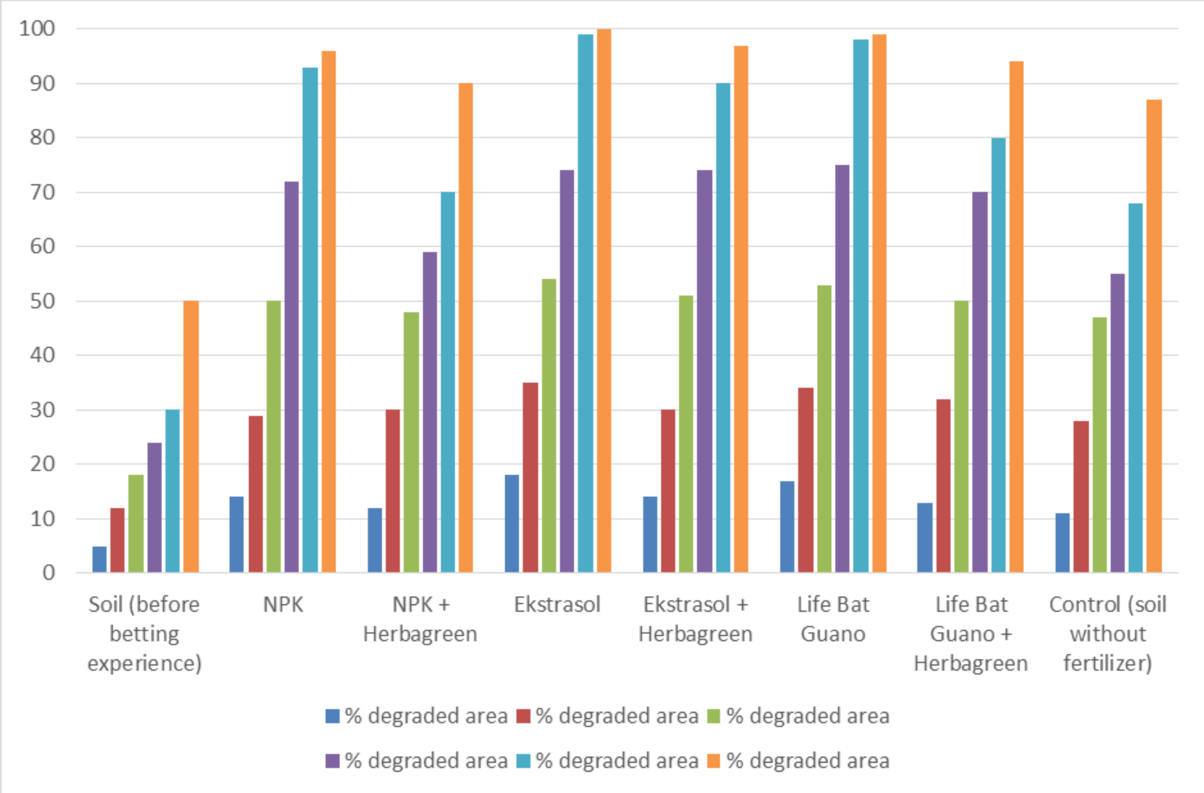

Figure 3: Cellulase Activity Of Soil Microorganisms

Lowest is the activity of the cellulase at the sample before beginning of the experiment, as well as at the control one, not fertilized soil (respectively 50 % and 87 % decomposed area). This result correlates with the lower content of the studied nutrient elements nitrogen, phosphorus and potassium at these samples in comparison with the fertilized ones. The bringing in of mineral fertilizers increases the activity of the enzyme with 40 up to 50 % at the fertilized variants, than at the soil before beginning of the experiment. The decomposed area of the filter paper, which shows the activity of the cellulase, varies between 90 and 100% at all soils with fertilizer, as complete decomposition is achieved at the sample, fertilized with the preparation Ekstrasol on 90-th day of the reporting. Next with highest activity of the enzyme are the samples, fertilized with the preparations Life Bat Guano and Ekstrasol + Herbagreen. It makes impression that the variants with these three preparations are and with highest content of potassium. Only with 1 % lower value of the decomposed area in comparison with the sample, fertilized with Ekstrasol + Herbagreen is the one, which contains NPK, which correlates with highest values of nitrogen and phosphorus at the soil with NPK, regarding the other variants. There is not established a clearly expressed tendency for increasing of the cellulase activity at the samples, fertilized with combined fertilizers in comparison with the other variants, as well as at the catalase activity.

4. CONCLUSIONS

Out of all used fertilizer products, the values of total potassium and phosphorus in the soil are highest at usage of Ekstrasol, and for the total nitrogen highest results are reported at the variants with mineral fertilizing.

The activity of the enzyme catalase is highest at the combined fertilizing (NPK + Herbagreen, Ekstrasol + Herbagreen and Life Bat Guano + Herbagreen) in comparison with the soils, at which is used one fertilizer product and the controls. Consequently, not only the better nutrient reserve with the nutrient elements at the fertilized, but also the combining of more than one preparation leads to increase of the activity of the catalase.

The fertilizing increases the activity of the cellulase at all variants in comparison with the control samples. This result correlates with the higher content of macroelements (nitrogen, phosphorus, potassium) at the fertilized soils in comparison with the control ones. The activity of the enzyme cellulase at the soil with brought in Ekstrasol is two times higher (100 % decomposed area), in comparison with the lowest activity at the sample before beginning of the experiment (50 % decomposed area). There is not established a clearly expressed tendency for increasing of the cellulase activity at the samples, fertilized with combined fertilizers in comparison with the rest variants, as at the catalase activity.

The activities of the enzymes catalase and cellulase are an important indicator for the biological condition of fertilized soils. They are indicative of the occurring biochemical changes at bringing in of fertilizer products in the soils.

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