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SOIL MICROBIOME UNDER THE INFLUENCE OF NANO AND BIOPREPARATIONS

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ABSTRACT

The results of the study of soil microbiome in Kent soybean crops under the influence of nanopreparation "Avatar 2 Organic" and biopreparations "Groundfix" and "Ecostern" are presented in the paper. It is shown that nano- and biopreparations can significantly affect the number of major groups of soil microorganisms. Features of exposure depend on the type of preparations and compositions in which they are used. It was found that the combined use of nano- and biopreparations inhibits the development of oligotrophs, cellulosic and sporeforming bacteria. As a result, there is a weakening of the destruction processes of organic matter and the predominance of its synthesis. It is shown that the nanopreparation "Avatar 2 Organic", subject to the recommended application standards, does not have a toxic effect on biopreparations that contain soil bacteria Bacillus subtilis, Enterobacter sp., Bacillus megaterium var phosphaticum, Enterococcus sp, Paenibacillus polymyxa, Azotobacter sp. The antimicrobial activity of the nanopreparation begins to appear after increasing the rate of its consumption by more than 100 times.

Key words: biopreparation, Ecostern, Groundfix, nanopreparation, soil, soil microbiome, physiological groups of microorganisms.

INTRODUCTION

Nanopreparations and biopreparations - elements of the modern technologies for growing crops, which have significant prospects for application. But some issues related to their effectiveness need further study. In particular, data on the effect of nanopreparations on the soil microbiome and, accordingly, on microorganisms that are part of biopreparations are contradictory. Features of the impact of nanopreparations on biological objects due to the unique physical and chemical properties of nanoparticles that are part of them: nanosize, shape, chemical composition, solubility, surface structure and aggregation (Arora et al., 2012; Kahru et al., 2010). A number of studies have shown that coating nanoparticles of biofertilizers, which include species of bacteria belonging to the genera: Rhizobium, Azotobacter, Azospirillum, Pseudomonas and Bacillus, helps to increase their tolerance to adverse

environmental conditions. In particular, gold nanoparticles stimulate the development of soil microorganisms that help increase the productivity of agricultural plants (Shukla et al., 2015).

According to McKee (McKee et al., 2016), nanopreparations can be toxic to soil organisms. They are able to bioaccumulate and be transported along trophic chains, affect the nitrogen and carbon cycle and other biogeochemical cycles in which microorganisms are involved. Nanopreparations can inhibit the activity of bacteria involved in the conversion of nitrogen compounds in the soil (Hegde et al., 2016). It is known that due to the interaction with biological systems, nanopreparations can change their characteristics. Due to active and passive transportation, cells of biological objects easily absorb nanoparticles, which are characterized by bactericidal action and are able to: damage cell membranes, oxidize proteins, interact with the respiratory chain and locally deplete protons, produce reactive oxygen species (ROS) (Hegde et al., 2015). The main depositor of nanoparticles is soil, where their concentration is much higher than in water or air (Dinesh et al., 2012). According to a retrospective analysis of the literature, the mechanism of influence of nanoparticles on both pathogenic and non-pathogenic microorganisms is the same. However, the degree of toxicity of nanoparticles to non-pathogenic microorganisms involved in nutrient mineralization and decomposition of organic carbon in the soil remains insufficiently studied.

Given the spread of environmentally friendly technologies for growing cultivated plants, the involvement of biological (microbiological) preparations deserves special attention. Microbiological preparations have been available on the market for a long time (Pylak et al., 2019). They are able to stimulate the biological activity of the soil, promote the development of soil microorganisms, improve plant growth and development (Jarecki et al., 2012). Therefore, to determine the impact of nanopreparations on biopreparationa of the new generation, as well as their combined effect on soil microbiota are relevant areas of research.

MATERIALS AND METHODS

The research was performed with the following preparations:

- nanopreparation "Avatar 2 Organic" (Avatar), which includes: nanocarboxylates of potassium, magnesium, boron, zinc, iron, copper, manganese, molybdenum, cobalt, vanadium, nickel, titanium, lanthanum, selenium, germanium, sulfur, iodine, silicon, silver and cerium, and purified water. The qualitative and quantitative composition of the nanopreparation has been studied in previous works (Hegde et al., 2016):
- microbiological preparations "Groundfix" and "Ecostern" (manufactured by LLC "BTU-Center"), which contain soil bacteria: *Bacillus subtilis, Enterobacter* sp., *Bacillus megaterium var phosphaticum, Enterococcus* sp, *Paenibacillus polymyxa, Azotobacter* sp.

The study was performed according to the following options:

1 - biopreparation Groundfix, 2 - biopreparation Ecostern, 3 - nanopreparation Avatar 2 Organic, 4 - a mixture of biopreparation Ecostern + nanopreparation Avatar 2 Organic, 5 - mixture of biopreparation Groundfix + nanopreparation Avatar 2 organic, 6 - control (water).

Concentrations of solutions were prepared in accordance with the recommended consumption rates:

Ecostern - 1.0 l ha^{-1}, Groundfix - 5.0 l ha^{-1}, Avatar 2 Organic - 150 ml ha^{-1}. The preparations were added to pots with soil at the rate of 157 µl of working solution per 1 pot with a volume of 300 ml. “Kent” soybean seeds were sown in the soil. In the plant germination phase, the soil microbiome was studied by the method of successive dilutions. According to the method, 10 g of soil was placed in a flask containing 90 ml of sterile tap water and shaken for 5 min. From the flask 1 ml of the soil suspension was transferred to a test tube containing 9 ml of sterile water, from this tube 1 ml of the suspension was transferred to the next with 9 ml of water. Thus, a series of consecutive dilutions were made. Suspensions of dilutions 103, 104 and 105 were sown on nutrient media (Volkohon et al., 2010). The total number of bacteria was determined on Zviahintsev's medium. Ashby medium was used to study Azotobacter, Saburo agar (SA) was used for yeast, starch-ammonia agar (SAA) was used for the total number of actinomycetes and microorganisms, hungry agar (HA) - for oligotrophs, and soil agar (SA) - for pedotrophs, Vynohradskyi 's environment with filter paper - for research of available quantity of cellulose-destroying bacteria. Sowing was done in three replicates, which were cultivated at a temperature of + 29° C for 24-48 hours. The results were calculated by weight of dry soil (State Standard of Ukraine. (2015). Soil quality. Determination of the number of microorganisms in the soil by sowing on a solid (agar) nutrient medium (Standard No. 7847). To determine the sensitivity of bacteria that are part of the biopreparations Groundfix and Ecostern to the nanopreparation Avatar, the method of holes was used. According to the method, 100 µl of biopreparation was applied to Zviahintsev's nutrient medium in accordance with the consumption rate specified by the manufacturer: "Ecostern" - 1.0 l ha^{-1}, "Groundfix"

- 5.0 l ha^{-1}. In the spilled medium, the holes were made with a punch, and then filled the bottom of the hole with a thin layer of medium. Dilution of the nanopreparation was prepared in accordance with the standards specified by the manufacturer on sterile tap water: "Avatar" – ml ha^{-1}. Volumes of 10, 100 and 1000 were calculated according to the recommended cost norms (RN). After that, 100 µl of the preparation "Avatar" was added to the hole according to the variants of the experiment. Incubation was carried out at a temperature of + 29 ° C for 24 hours. The calculations were performed by measuring the diameter of the growth retardation zone of the biopreparation (Kuleshova, 2015). The antimicrobial effect of nanopreparation Avatar on biopreparationswas set according to the following gradation:

Toxicity level:	Growth retardation zone, mm:
low	5-10
medium	10-20
high	>20

Statistical analysis

The significance of the experimental data was estimated by the analysis of variance (two-factor, ANOVA).

RESULTS AND DISCUSSION

The results of the study showed that nano- and biopreparations have a significant impact on the number of major groups of soil microorganisms (Table 1, Fig. 1). The total number of microorganisms that use mainly organic nitrogen compounds during the application of the nanopreparation Avatar was $6.6 \cdot 10^5$ CFU/g of soil. At the same time, under the influence of biopreparations, it ranged from 4.0 to $4.6 \cdot 10^5$ CFU/g of soil. In the control variant, the number of tested microorganisms was $2.2 \cdot 10^5$ CFU/g of soil. The combined use of nano- and biopreparations has led to a significant increase in this group of soil microorganisms. Their number increased to $2.3\text{-}4.4 \cdot 10^6$ CFU/g of soil. The most effective was the combined use of nanopreparation Avatar and biopreparation Groundfix where the number of microorganisms increased to $4.4 \cdot 10^6$ CFU/g of soil (Fig.1, Table 1).

Table1. Influence of nano- and biopreparations on the number of main physiological groups of soil microorganisms (Kent soybean)

Group of microorganisms	Number of microorganisms, CFU per 1 g of dry soil					
	Sample					
	1	2	3	4	5	6
The total number of microorganisms that use mainly organic nitrogen compounds	4.6×10^5	4.0×10^5	6.6×10^5	2.3×10^6	4.4×10^6	2.2×10^5
Yeast	1.3×10^5	7.0×10^3	1.8×10^4	6.2×10^4	3.0×10^3	1.0×10^3
Azotobacter	7.0×10^4	8.8×10^4	1.3×10^5	2.4×10^5	5.6×10^4	5.9×10^4
Oligotrophs	1.3×10^5	1.4×10^5	2.9×10^5	3.0×10^4	1.1×10^5	5.8×10^4
Cellulose-destroying	1.9×10^3	2.7×10^3	4.0×10^3	2.3×10^3	2.8×10^3	1.0×10^3
Pedotrophic	2.5×10^5	1.6×10^5	2.6×10^5	5.8×10^5	2.7×10^6	2.4×10^5
Microorganisms that use mainly min. nitrogen compounds	7.0×10^4	2.0×10^4	2.0×10^5	1.2×10^5	1.1×10^6	1.6×10^5
Actinomycetes	5.1×10^4	3.5×10^4	4.8×10^4	6.0×10^4	1.1×10^5	5.8×10^4
Sporeforming	2.2×10^4	9.7×10^5	9.2×10^4	1.5×10^5	1.9×10^5	4.0×10^4

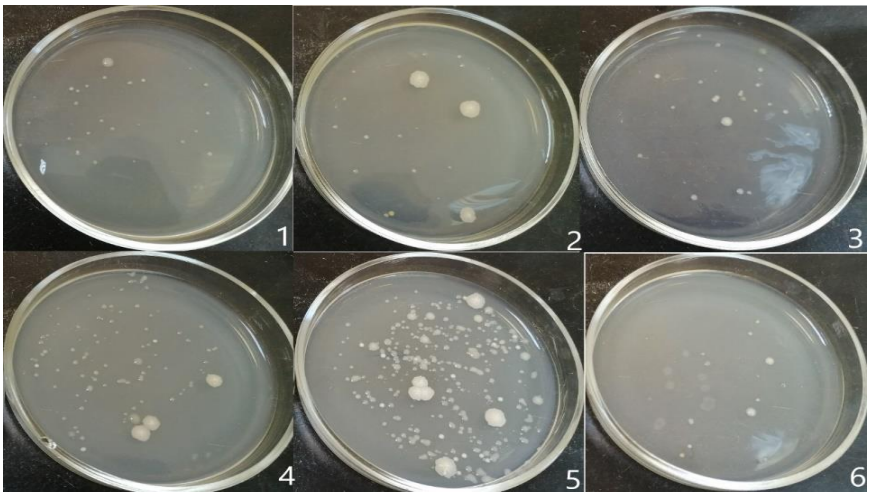

Figure 1. Colonies of microorganisms using mainly organic nitrogen compounds (Zviahintsev's medium, dilution 10^5):
1 - biopreparation Groundfix, 2 - biopreparation Ecostern, 3 - nanopreparation Avatar 2 Organic, 4 - mixture of biopreparation Ecostern + nanopreparation Avatar 2 Organic, 5 - mixture of biopreparation Groundfix + nanopreparation Avatar 2 Organic, 6 - control (water).

The combined use of the nanopreparation Avatar with the biopreparation Graunfix also increased (up to $1.1 \cdot 10^6$ CFU/g of soil) the number of microorganisms that use mineral nitrogen compounds. Biopreparations Graunfix and Ecostern increased to $7.0 \cdot 10^4$ CFU/g of soil and to $2,0 \cdot 10^4$ CFU/g of soil respectively, the number of nitrogen-fixing bacteria. This indicates the inclusion of mineral nitrogen in the metabolism of microorganisms, which creates conditions for their competition for this element (Kolomiyets et al., 2016; Symochko, 2020).
Under the influence of nano- and biopreparations, the number of oligotrophic microorganisms increased. The increase in the number of oligotrophs indicates the accessibility of easily digestible substances that accumulate during the transformation of organic residues, including plant. This helps to improve trophic connections in the structure of the soil microbial complex (Symochko et al., 2008). The highest values of the number of oligotrophs were observed when using the nanopreparation - $2.9 \cdot 10^5$ CFU/g of soil, biopreparations also contributed to the increase in the number of this group of microorganisms - their number was at the level of $1.3\text{-}1.4 \cdot 10^5$ CFU/g of soil, on control - $5.8 \cdot 10^4$ CFU/g of soil. However, the combined use of these preparations reduced the number of oligotrophs to $3.0\text{-}11,0 \cdot 10^4$ CFU/g of soil (Fig. 2).

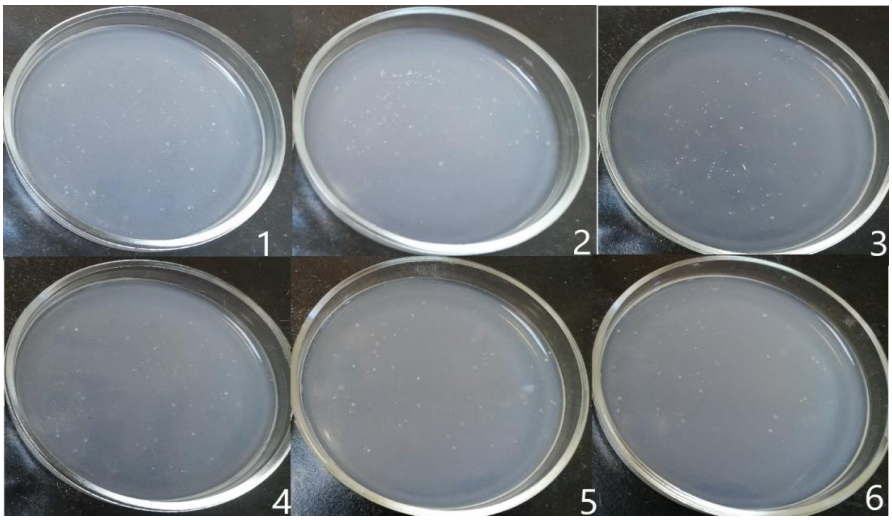

Figure 2. Colonies of oligotrophic microorganisms (Pure Agar medium, dilution 10^4):
1-biopreparation Groundfix, 2-biopreparation Ecostern, 3 - nanopreparation Avatar 2 Organic, 4 - mixture of biopreparation Ecostern + nanopreparation Avatar 2 Organic, 5 - mixture of biological product Groundfix + nanopreparation Avatar 2 Organic, 6 - control (water)

The number of cellulose-destructive microorganisms that actively function in the microbiocenosis in the initial stages of transformation of organic matter (Patyka et al., 2014) did not change significantly under the influence of nano- and biological products, and ranged from 1.0 to $4.0 \cdot 10^3$ CFU/g of soil. However, there was a tendency to increase them relative to control, especially with the use of nanopreparation Avatar (Table 1, Fig.2).
The biopreparation Ecostern caused a significant increase in spore-forming bacteria. Their number reached the level of $9.7 \cdot 10^5$ CFU/g of soil (in control – $4{,}0 \cdot 10^4$ CFU/g of soil). This indicates the active participation of the above-mentioned physiological group in the destruction of organic residues, namely soil purification (Patyka et al., 2014). The combined use of the biopreparation Ecostern with the nanopreparation Avatar led to a decrease in the number of these bacteria to $1.5 \cdot 10^5$ CFU/g of soil (Fig. 3). The use of Groundfix biopreparation both separately and in combination with the Avatar nanopreparation reduced the number of spore-forming bacteria relative to control.

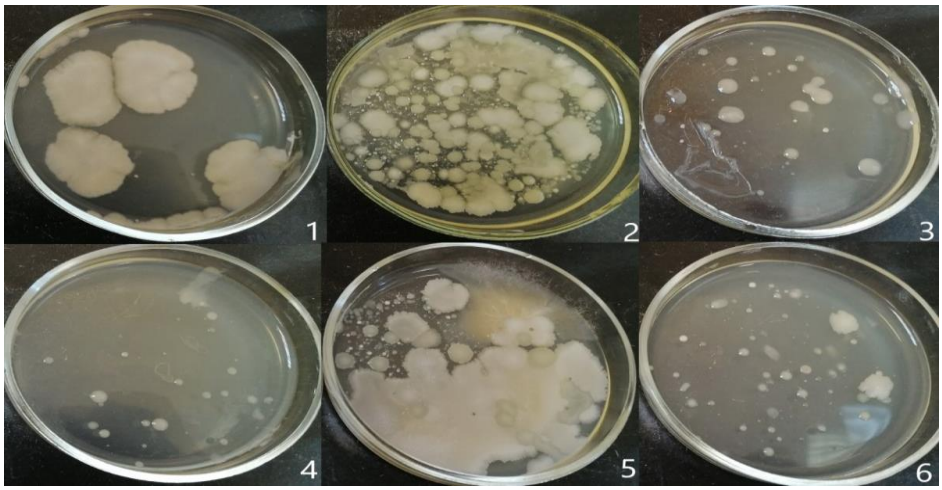

Figure 3. Colonies of spore-forming microorganisms (Zviahintsev's medium, dilution 10^4):
1 - biopreparation Groundfix, 2 - biopreparation Ecostern, 3 - nanopreparation Avatar 2 Organic, 4 - mixture of biopreparation Ecostern + nanopreparation Avatar 2 Organic, 5 - mixture of biopreparation Groundfix + nanopreparation Avatar 2 Organic, 6 - control (water).

Nitrogen-fixing bacteria in the soil samples studied were mainly species of the genus *Azotobacter*, which are able to absorb atmospheric nitrogen from the air and in the process of life to form proteins and other organic nitrogen compounds from molecular nitrogen used by plants. The use of biopreparations Groundfix and Ecostern increased the number of species of the genus *Azotobacter* to the level of 7.0-8.8 $\cdot 10^4$ CFU/g of soil (on control 5.9 $\cdot 10^4$ CFU/g of soil), nanopreparation Avatar – до $1.3 \cdot 10^5$ CFU/g of soil. Combined use of Ecostern with Avatar contributed to a further increase in the number of *Azotobacter* ($2.4 \cdot 10^5$ CFU/g of soil), and Groundfix - on the contrary, reduced the number of nitrogen-fixing bacteria (5.6 $\cdot 10^4$ CFU/g of soil). Thus, the nanopreparation Avatar stimulates the development of microorganisms that use organic compounds, oligotrophs, yeast, cellulose-destroying and spore bacteria, nitrogen bacteria. Combined use of nanopreparation Avatar and biopreparation Graunfix has a positive effect on the development of microorganisms that use organic and mineral compounds of nitrogen, actinomycetes, pedotrophs. Combined use of the nanopreparation Avatar with the biopreparation Ecostern increases the number of microorganisms that use organic compounds of nitrogen, yeast, actinomycetes, nitrogen bacteria. Combined use of nanopreparation Avatar with biopreparations Biofix and Ecostern inhibits the development of oligotrophs, cellulose-destroying and spore bacteria. To determine the sensitivity of bacteria that are part of biopreparations, the method of wells was used to nanopreparation Avatar. This method allowed to estimate the level of antimicrobial activity of the nanopreparation in concentrations corresponding to 1, 10, 100 and 1000 recommended for the production of its application rates. It was found that increasing the application rate of nanopreparation Avatar from 1 to 100 recommended rates (RR) does not have a bactericidal effect on microorganisms that are components of biological products. Preparations can be used together in crop production technologies without reducing their effectiveness. Toxic effect of nanopreparation Avatar was appeared only when increasing the rate of its application to 1000 PH (in terms of 150 l ha^{-1}). At this concentration of nanopreparation bactericidal and bacteriostatic effect was manifested on the microbial component of the biopreparation Ecostern and bacteriostatic effect on microorganisms Groundfix, when diluting biological products in accordance with consumption rates (Fig. 5, Table 2).

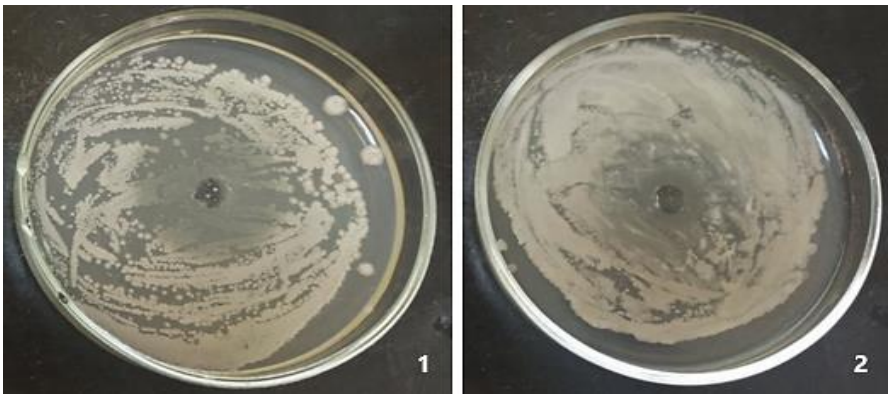

Figure 5. Antimicrobial action of nanopreparation Avatar (1000 RN) on microorganisms of biopreparations: 1 - Ecostern (1 l ha^{-1}), 2 - Groundfix (5 l ha^{-1}).

Table 2. Antimicrobial action of nanopreparation Avatar on microorganisms and biopreparations Ecostern and Groundfix, in dilution according to consumption norms.

Variant	Growth retardation zone (d, mm)	The presence of antimicrobial action, the possibility of combined use
Ecostern	0	-
Ecostern + 1 RR Avatar	0	absent, preparations are compatible
Ecostern + 10 RR Avatar	0	absent, preparations are compatible
Ecostern + 100 RR Avatar	0	absent, preparations are compatible
Ecostern + 1000 RR Avatar	18 b/c 31 b/s	toxicity is medium, bacteriostatic effect of the preparations is manifested, preparations are partially compatible
Groundfix	0	-
Groundfix + 1 RR Avatar	0	absent, preparates are compatible
Groundfix + 10 RR Avatar	0	absent, preparates are compatible
Groundfix + 100 RR Avatar	0	absent, preparates are compatible
Groundfix + 1000 RR Avatar	28.7 b/s	bacteriostatic action of nanopreparate is shown, preparates are partially compatible

Notes: "0" - no bactericidal action; "Digital mark" - the diameter in mm of the area of action of the drug; "BC" - bactericidal action; "BS" - bacteriostatic action.

Thus, the results of the study showed that in the conditions of production subject to compliance with the regulations of application (compliance with application standards), nanopreparation Avatar will not have a negative effect on the microbial components such us: *Bacillus subtilis, Enterobacter, Bacillus megaterium var phosphaticum, Enterococcus, Paenibacillus polymyxa, Azotobacter.*

CONCLUSIONS

- It is established that nano- and biopreparations can significantly affect the number of major groups of soil microorganisms. Peculiarities of influence depend on the type of preparations and compositions in which they are used.
- It is shown that the nanopreparation Avatar can stimulate the development of microorganisms from groups that use organic compounds of nitrogen, oligotrophs, yeast, cellulose-destroying and spore bacteria,

azotobacter. Its combined use with the biopreparation Graunfix can increase the number of microorganisms that use organic and mineral compounds of nitrogen, actinomycetes, pedotrophs; with biopreparation Ecostern - the number of microorganisms that use organic compounds of nitrogen, yeast, actinomycetes, azotobacter.

- It was found that the combined use of nanopreparation Avatar with biologic products Groundfix and Ecostern inhibits the development of oligotrophs and sporeforming bacteria.
- It is established that the use of nano- and biopreparations, both separately and in combination, led to a weakening of the destruction of organic matter and the predominance of its synthesis. Using of Avatar, Ecostern and Groundfix slows down the intensity of decomposition of organic matter.
- It is shown that the nanopreparation Avatar, subject to the recommended application standards, will not have a toxic effect on biopreparations that contain soil bacteria of the genus *Bacillus subtilis*, *Enterobacter*, *Bacillus megaterium var phosphaticum*, *Enterococcus*, *Paenibacillus polymyxa*, *Azotobacter*.

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