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Potentially Beneficial *Comamonas Testosteroni* Bacteria for Plants Growing in HCB-Polluted Soil

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Abstract

The bioaugmantation effect of bacterial strains on plant development in organochlorine pesticides (OCP) polluted soil has been in the focus of attention, however there is little information about *Comamonas testosteroni* strains influence. The investigation was performed by classic methods. The results of the research showed that *Comamonas testosteroni* UCM B-400 and B-401has a high destroying potential to xenobiotics in the soil, and also has a positive effect on plant development. The tomato plants ability to develop under conditions with *Comamonas testosteroni* UCM B-400 and B-401 bioaugmentation in HCB - contaminated and uncontaminated soil were studied. *Comamonas testosteroni* UCM B-400 and B-401 promoted the increasing of photosynthetic activity, biometric parametrs and increase the resistance to phytopathogens, such as Clavibacter michiganensis subs. michiganensis UCM Ac-629 (up to 36%) and Alternaria alternata UCM F-16866 (up to 32%). *Comamonas testosteroni* UCM B-400 and B-401 can be used as an inoculant to improve plant development conditions in HCB load soils.

Keywords: *Comamonas Testosteroni;* Hexachlorobenzene; Tomatoes Cultivar "Lagidniy"

Abbreviations: OCP: Organochlorine Pesticides; HCB: Hexachlorobenzene; CFUs: Colony-Forming Units; PAHs: Polycyclic Aromatic Hydrocarbons.

Introduction

Bioremediation of pesticide-polluted soil ecosystems via themicroorganisms - xenobiotics potential destructors has significant advantages in application compared to chemical and physical methods due to safety, economy and prolonged effect. Bioaugmentation is one of the most effective methods of bioremediation [1]. Due to the intensive activity of agro-industrial production, a large amount of pesticides has accumulated in the soil that need to be removed. One of the dangerous toxicants is a group of organochlorine pesticides, which includes hexachlorobenzene (HCB), which is prohibited by the Stockholm Convention [2]. Bacteria *Comamonas testosteroni* are known to be destructors of aromatic compounds, such as nitrobenzenes, chlorobenzenes and pentachlorophenol [3-5]. Due to the high destructive potential of these bacteria, many studies have been conducted as remediates of contaminated soils. The using *Comamonas* strains were carryed out in combination with plants, such as alfalfa [6], as well as with Bacillus sp. bacteria [5,7] and as a monoculture [8,9] for the removal of xenobiotics from soils. In all cases, high remediation capacity of the studied

bacteria was reported. Studying *Comamonas*-alfalfa system was applied for remediation of *tetrachloronitrobenzene* (4CNB) polluted soil demonstrated 4CNB degradation and 4CNB phytotoxicity elimination by *Comamonas sp*. strain CNB-1 [6].

The capacity of *Bacillus subtilis* strain DKT and *Comamonas testosteroni* KT5 to fomate the biofilm and degradate chlorobenzenes and toluenes were determined. Studying the dual-species biofilm of *C. testosteroni* KT5 and *B. subtilis* DKT demonstrated high degradability effect of chlorobenzene and 2-chlorotoluene and the possibility to remove from polluted ecotops mixture of toxic organic compounds that cannot be metabolized by single-organism biofilms [5]. Biodegradation of pentachlorophenol (PCP) by *Comamonas testosteroni* CCM 7530 in three chernozem soil types was investigated and showed the PCP content decreasing in soil, thatprovided reducing toxic effect for resident microbiotanumber, as soil quality and fertility characteristic [8]. Therefore, researchers have shown the ability of *Comamonas testosteroni*bacteria to destroy chlorobenzenes and other aromatic compounds, as well as reduce the toxic effect on microbiota and plants in the soil. It is important to study the effect as potentially hexachlorobenzene (HCB) degrading *Comamonas testosteroni* strains on plant development and their resistance to biotic factors. The aim of the study was to determine the effectiveness of the introducing *Comamonas testosteroni* strains UCM B-400 and UCM B-401 on the developing tomato plants and their resistance to phytopathogens under cultivating conditions in HCB-contaminated gray podzolic soil.

Methods

Experiment Design

The experiment was performed in the laboratory conditions in the following variants: 1 - uncontaminated soil; 2, 3 - introducing the *C. testosteroni* UCM B-400 and B-401 culture liquidinto the unpolluted soil; 4,5-HCB contaminated soil at doses of 30 and 100 mg / kg; 6, 7 - HCB loaded soil at 30 mg / kg doses, which which was inoculated by *C. testosteroni* UCM B-400 and *C. testosteroni* UCM B-401 culture liquid, respectively; 8 and 9 - HCB contaminated soil at 100 mg / kg doses, which was inoculated by *C. testosteroni* UCM B-400 and *C. testosteroni* UCM B-401 culture liquid. Tomato plants of the cultivar «Lagidniy» were grown in all experimental variants. Biometric parameters were determined at the 3-4 leaf formation stage. Resistance to phytopathogens was studied by the Krayntsburg-Eggert method under artificial infection conditions of leaf plates with the micromycete *Alternaria alternata* UCM F-16866 and bacteria *Clavibacter michiganensis* subs. michiganensis UCM Ac-629.

Microbial Quantity

The microorganisms number was assessed by sowing ten-fold dilutions of the soil suspension on agarified nutrient media and assessing the colony-forming units (CFUs) amount per gram of dry soil, taking into account its moisture content. The selective media used to determine certain ecological-trophic group have been described previously [10].

Biometric Parameters

There are plant length, root length, root mass, plant mass were determined at the 3-4 leaf formation stage.

Chlorophyll Content Measurement

Leaf samples were crushed and grinded with a pestle in a mortar. Extractions were done using 96% ethanol. The extract aliquots were used to spectrophotometrically determine total chlorophyll and chlorophylls a and b contents. Absorbance readings were carried out at 649, 654 and 665 nm, and the results are expressed as mg of chlorophyll/ g leaf tissue. Equations for calculate chlorophyll content are following: Chl a = $13.70\times D_{665} - 5.76\times D_{649}$; Chl b = $25.80\times D_{649} - 7.60\times D_{665}$; Total Chl (Chl a + Chl b) = $6.10\times D_{665} + 20.04\times D_{649} = 25.10\times D_{654}$ [11].

Resistance to Phytopathogens

Determination of tomatoes leaves infestation level was conducted by the Krayntsburg-Eggert method under artificial infection conditions of leaf plates with the micromycete *Alternaria alternata* UCM F-16866 and bacterium *Clavibacter michiganensis* subs. michiganensis UCM B-629.

Statistical Analysis

Data are presented as means ± SEM. Statistical analysis were performed by using GraphPad Prism 8.0.1 software. The data were analyzed with Student's t-test and $P \leq 0.05$ was considered to indicate significance difference. Significant differences between variants and the control are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$.

Results

The ecology-functional number of soil microbial groups made it possible to assess the soil quality andbiochemical processes direction in which the tomato plants ofcultivar «Lagidnii» developed. The positive effect of *Comamonas testosteroni* strains UCM B-400 and B-401 culture liquid

introducing into the soil was demonstrated in all structural-functional and systematic groups of microbiocenoses. The results obtained (Table 1) showed relative sensitivity to HCB, especially in the version with a higher toxicant dose. However, in contaminated soil, but with following bioaugmentation of *Comamonas testosteroni* UCM B-400 and B-401, there were a certain increase in the number of most ecology-functional groups inmicrobiocenoses, which suggests the destructive xenobiotics processes, namely HCB. Pedotrophic microorganisms to be representatives of the indigenous soil microbiota, showed the degree of microbialdevelopment and showed an increase in the number and, consequently, soil quality under conditions of HCB contamination with following introducing *Comamonas testosteroni* UCM B-400 and B-401 culture liquid. However, the quantity value of this group was lower compared to the uncontaminated control, but the amount inhancing dynamics of populationwas observed. Increasing the ammonifying bacteria amount showed the nitrogen mineralization intensity in the soil, which also improved its quality. This in the variant with bioaugmentation in uncontaminated soil was demonstrated.

Nitrogen-fixing and oligonitrophilicand amylolytic bacteria take part in the nitrogen cycle, the value of which reflects the nitrogen balance in the soil, which is very important for plant development. The oligotrophic bacteria number capable to grown on poor substrates, in all variants where culture liquids of the studied strains were introduced, decreased significantly, as emphasizing the active processes of organic matter transformation. Soil streptomycetes are known to be producers of important biochemical compounds for plant development. Micromycetes are also involved in humus formation, which isdirect indicator of soil fertility. Thus, the number of ecology-functional groups showed a positive trend in the development of microbiocenosis, which had a positive effect on plant development.

Microbial group	1	2	3	4	5	6	7	8	9
P	$5.3\pm05\times10^{7}$	$3.6\pm0.2\times10^{7**}$	$3.7\pm0.2\times10^{7**}$	$6.7\pm0.3\times10^{7*}$	$7.1\pm0.3\times10^{7**}$	$4.4\pm0.1\times10^{7*}$	$4.4\pm0.3\times10^{7*}$	$3.9\pm0.2\times10^{7**}$	$4.0\pm0.2\times10^{7*}$
Amm	$3.9\pm0.2\times10^{7}$	$2.7\pm0.3\times10^{7**}$	$3.3\pm0.2\times10^{7*}$	$4.5\pm0.2\times10^{7*}$	$5.4\pm0.5\times10^{7**}$	$2.9\pm0.4\times10^{7*}$	$5.0\pm0.7\times10^{7*}$	$4.5\pm0.2\times10^{7*}$	$4.6\pm0.1\times10^{7*}$
Amy	$3.0\pm0.2\times10^{7}$	$2.6\pm0.5\times10^{7*}$	$2.5\pm0.1\times10^{7*}$	$4.2\pm0.5\times10^{7*}$	$3.6\pm0.2\times10^{7*}$	$3.9\pm0.3\times1^{07*}$*	$3.6\pm0.2\times10^{7*}$	$3.9\pm0.3\times10^{7**}$	$3.9\pm0.2\times10^{7**}$
N O-nitrophil	$3.6\pm0.2\times10^{7}$	$2.7\pm0.2\times10^{7**}$	$2.6\pm0.2\times10^{7**}$	$5.0\pm0.6\times10^{7**}$	$5.6\pm0.7\times10^{7*}$	$3.9\pm0.01\times10^{7**}$	$4.1\pm0.1\times10^{7*}$	$4.8\pm0.2\times10^{7**}$	$3.9\pm0.01\times10^{7*}$
Ph	$1.2\pm0.06\times10^{6}$	$0.8\pm0.07\times10^{6**}$	$0.7\pm0.05\times10^{6***}$	$1.6\pm0.07\times10^{6**}$	$1.5\pm0.1\times10^{6**}$	$1.0\pm0.05\times10^{6*}$	$0.7\pm0.2\times10^{6*}$	$0.8\pm0.07\times10^{6**}$	$0.8\pm0.07\times10^{6**}$
Oligotr	$3.4\pm0.1\times10^{7}$	$2.6\pm0.2\times10^{7**}$	$3.0\pm0.2\times10^{7**}$	$1.5\pm0.01\times10^{7*}$	$1.3\pm0.1\times10^{7**}$	$1.4\pm0.1\times1^{07*}$*	$1.3\pm0.01\times10^{7****}$	$1.2\pm0.01\times10^{7****}$	$1.2\pm0.1\times10^{7**}$
S	$1.6\pm0.4\times10^{5}$	$1.0\pm0.01\times10^{5*}$	$0.9\pm0.1\times10^{5*}$	$3.0\pm0.3\times10^{5**}$	$2.9\pm0.5\times10^{5*}$	$2.7\pm0.3\times10^{5*}$	$2.5\pm0.2\times10^{5*}$	$2.7\pm0.4\times10^{5*}$	$2.4\pm0.2\times10^{5*}$
M	$4.8\pm0.5\times10^{4}$	$3.9\pm0.2\times10^{4*}$	$3.3\pm0.3\times10^{4*}$	$3.9\pm0.01\times10^{4*}$	$4.0\pm0.4\times10^{4***}$	$3.5\pm0.3\times10^{4*}$	$3.1\pm0.4\times10^{4**}$	$3.2\pm0.3\times10^{4*}$	$3.2\pm0.6\times10^{4*}$

Table 1: Microbial amount in HCB- polluted soil with/out biaugmentation.
P – Pedotrophics, Amm – Ammonifyings, Amy – Amylolytics, N+Onitrophil – Nitrogen-fixing and Oligonitrophilics, Ph – Phosphate-mobilizings, Oligotr – Oligotrophics, S – Streptomycetes, M – Micromycetes;1 – uncontaminated soil; 2 – 30 mg/kg HCB-contaminated soil; 3 – 100 mg/kg HCB- contaminated soil; 4 – introducing *C. testosteroni* UCM B-400; 5 – introducing*C. testosteroni* UCM B-401 culture liquid into unpolluted soil; 6 – 30 mg/kg HCB-loaded soil followed by inoculating *C. testosteroni* UCM B-400 culture liquid;7 – 30 mg/kg HCB-loaded soil followed by inoculating *C. testosteroni* UCM B-401 culture liquid;8 – 100 mg/kg HCB-contaminated soil followed by inoculating *C. testosteroni* UCM B-400 culture liquid 9 – 100 mg/kg HCB-contaminated soil followed by inoculating *C. testosteroni* UCM B-401 culture liquid. Notes: Significant differences between variants and the control are indicated by *p < 0.05; **p < 0.01 and ***p < 0.001.

The obtained results demonstrate that introduction of studied strains in polluted soil exert the phytostimulating effect on plants, which is confirmed by the increasing of all biometric parameters, especially plant mass: by 17.5 and 20%, respectively for strains B-400 and B-401. In plants growing in HCB contaminated soil, growth inhibition was observed, it was most pronounced in the variants with 100 mg / kg HCB: the plant mass was decreased by 46% compared to control plants without HCB-loading (Figure 1).

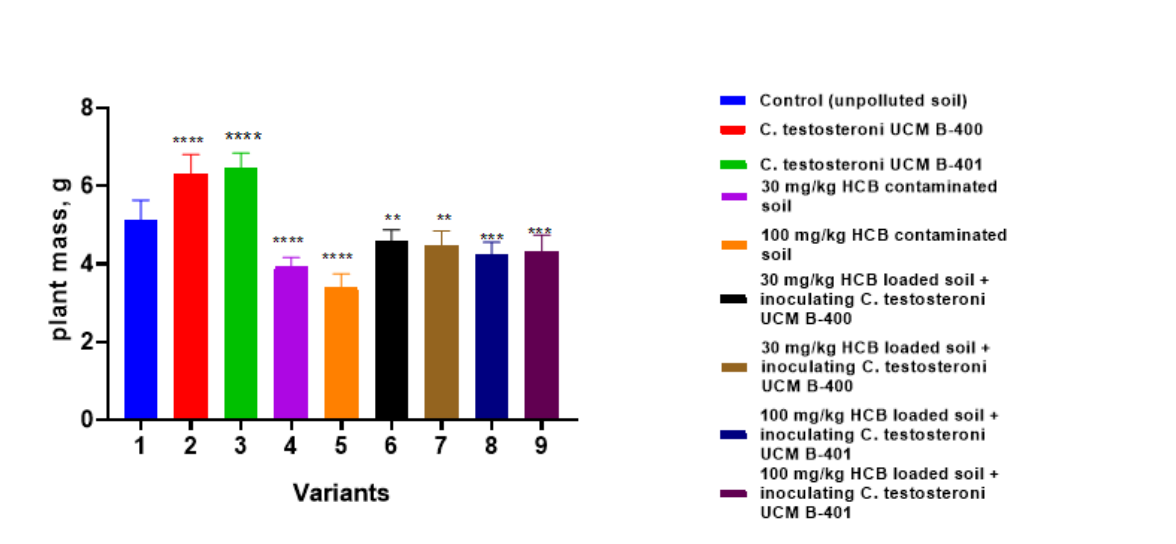

Figure 1: Effect on plant mass after growing in soil with *Comamonas testosteroni* strains introducing compared to unpolluted and HCB-polluted soil. Notes: Significant differences between variants and the control are indicated by *p < 0.05; **p < 0.01 and ***p < 0.001.

The plant length increased to 15 –18% compared to control. Also, in the variants from polluted soil, the plant length decreased by 29 and 33%. At the same time, in the variants with biougmentation, this value decreased by 3 – 8% (Figure 2).

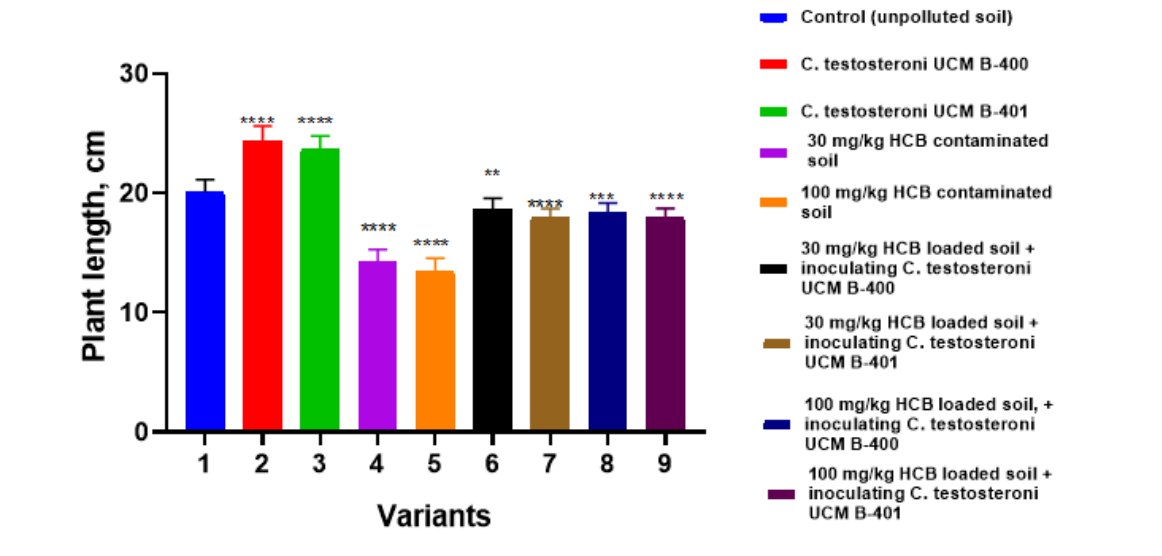

Figure 2: Effect on plant length after growing in soil with *Comamonas testosteroni* strains introducing compared to unpolluted and HCB-polluted soil. Notes: Significant differences between variants and the control are indicated by *p < 0.05; **p < 0.01 and ***p < 0.001.

The maximum increase in root length, which was 10% in the variant with the introduction of *C. testosteroni* UCM B-400 without contamination was observed. The greatest suppression by this indicator - 27%in the variant with 100 mg / kg HCB polluted soil was observed (Figure 3).

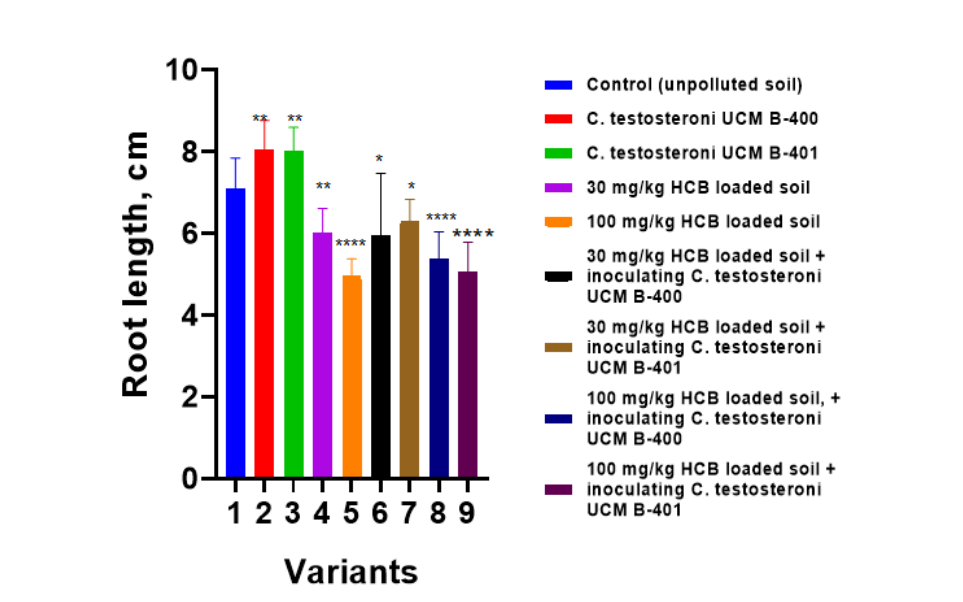

Figure 3: Effect on root length after growing in soil with *Comamonas testosteroni* introducing compared to unpolluted and HCB-polluted soil. Notes: Significant differences between variants and the control are indicated by *p < 0.05; **p < 0.01 and ***p < 0.001.

The *C. testosteroni* culture liquid introducing into the polluted soil reduced the negative impact of the pesticide load. Thus, compared to plantsgrowing in contaminated soil withoutbacterial introducing, under using *C. testosteroni* UCM B-400, the root mass increased by 19 and 24%, respectively, for 30 and 100 mg / kg HCB. Using*C. testosteroni* UCM B-401 strain increased the roots mass, in the variant with 30 mg / kg up to 24.4%, and in the variantwith 100 mg / kg HCB up to 14.6% (Figure 4).

Nevertheless, all biometric indicators were lower to 7.5 – 23% compared to plants growing in unpolluted soil.

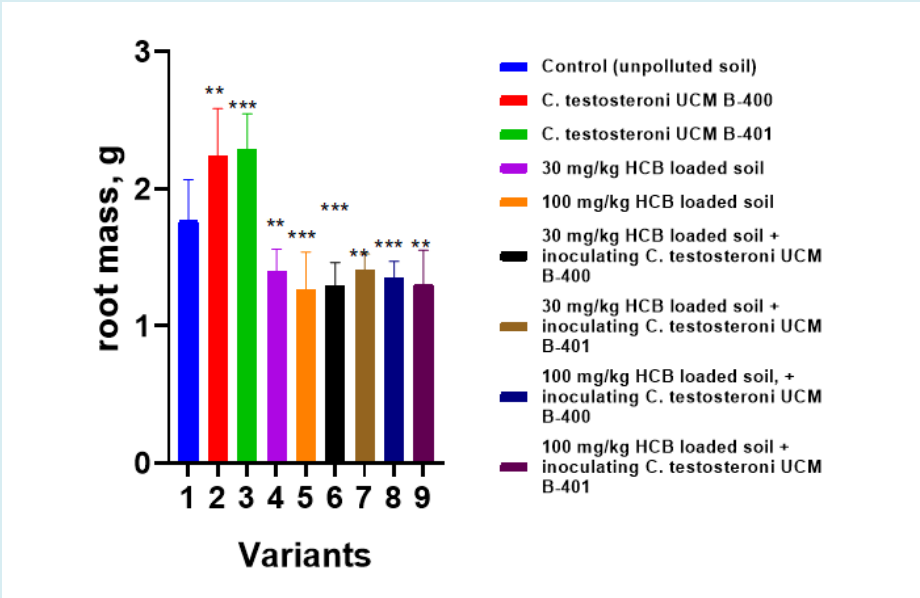

Figure 4: Effect on root length after growing in soil with *Comamonas testosteroni* introducing compared to unpolluted and HCB-polluted soil. Notes: Significant differences between variants and the control are indicated by *p < 0.05; **p < 0.01 and ***p < 0.001.

Chlorophyll content is one of the main indicators of the leaf ability to photosynthesis and plant health. Under conditions with *Comamonas testosteroni* biougmentation, the level of chlorophyll increased by 65%, which indicates an inhance in the photosynthesis level. However, under conditions with HCB contamination, the chlorophyll content decreased by almost 40% compared to the control. Under the conditions of tomato germination in HCB-loaded soil with following inroducing *C. testosteroni*, the level of chlorophyll was equality to control plants from uncontaminated soil. That is, *Comamonas testosteroni* UCM B-400 and B-401 promotes stress resistance of plants, and on the other hand reduces the pesticide load, which also improves the conditions for plant development.

Regarding the tomatoes resistance level to phytopathogens, it nesessery to note that it was observed both to *Alternaria alternata* UCM F-16866 and to *Clavibacter michiganensis* subs. *michiganensis* UCM Ac-629. Under conditions of inoculating *C. testosteroni* strains, resistance to *Clavibacter michiganensis* increased to 28 – 36% compared to control. In variants with HCB loading followed by thebacterial suspensions introducing, an increase in resistance to *Clavibacter michiganensis* UCM B-629 to 8 – 16% was observed. Resistance to *Alternaria alternata* UCM F-16866 up to 32% in variants with biougmentation without contamination was observed. Also, tomato leaves resistance in variants with bioaugmentation of HCB-contaminated soil, up to 20 – 28% compared to control was observed.

Discussion

The studing results showed a positive effect of two*C. testosteroni* strains on plant development. Determining the number of ecology-functional groups in soil microbiocenosis showed that *C. testosteroni* influences its structure. Thus, a study Sun X, et al. [12] reported the introducing these bacteria into soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and as a result showed that *C. testosteroni* increases the interaction between bacteria capable to destroy PAHs. Gentry and co-authors Tan WA, et al. [13] studied the effectiveness of bioaugmentation of *C. testosteroni* in 3-chlorobenzoate contaminated soil and showed that the target strains not only reduced the toxicant content but also reduced the negative impact on the resident soil microbiota. Theour study results also showed a change in the structure of the microbiocenosis after the introducing strains into the soil, even contaminated with HCB, which promoted to improving soil quality.

Khalofah and co-authors Vítková, et al. [14] showed a positive effect on the development of *Linum usitatissimum* L. plants under oxidative stress. *C. testosteroni* increased the level of photosynthetic pigments. *C. testosteroni* also promoted to the formation of soluble sugars, proline and soluble proteins, and oxidative stress levels such as H2O2 and malonic dialdehyde decreased.*C. testosteroni* bacteria were isolated from durum wheat fields [15] and after studying their biological properties, found that they are able to transform insolublephosphate forms, potassium and zinc and were recommended as inoculants to increase plant productivity. Our study also showed an increase in biometric indicators of tomatoes, as well as chlorophyll in the presence of *C. testosteroni* bacteria in the soil [16].

Conclusions

- Biougmentation of C. testosteroni UCM B-400 and B-401 strains into the HCB-polluted soil improves conditions for plant development, has a phytostimulating and protective effects on tomatoes of cultivar “Lagidniy”.
- The C. testosteroni B-400 and B-401 strains culture liquid introducing into the HCB-polluted soil reduced the negative impact of the pesticide load on tomato plants.
- The introducing bacterial cultures liquids into the HCB-loaded soil increased the plant resistance to phytopathogens Alternaria alternata UCM F-16866 and Clavibacter michiganensis subs. michiganensis UCM Ac-629.

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