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Biological effectiveness of a new multifunctional biopesticide in the protection of organic potatoes from diseases

I.I. Novikova[1], V.B. Minin[2,*], J.A. Titova[1], I.L. Krasnobaeva[1], A.M. Zaharov[2] and A.N. Perekopsky[2]

[1]Federal State Budget Scientific Institution "All-Russian Research Institute of Plant Protection" (FSBSI VIZR), 3, Podbelskogo shosse, RU196608 Saint-Petersburg-Pushkin, Russia
[2]Institute for Engineering and Environmental Problems in Agricultural Production branch of the BFSBSI "Federal Scientific AgroEngineering Centre VIM (IEEP – branch of FSAC VIM), 3, Filtrovskoe shossse, p.o. Tiarlevos, RU196625 Saint-Petersburg, Russia
[*]Correspondence: minin.iamfe@mail.ru

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Abstract. Crop disease control is of particular importance in organic crop production, as the use of chemical pesticides is prohibited there. A new multifunctional biofungicide Kartofin was selected and used to optimize the phytosanitary state of organic potatoes ecosystems. Previously of studies indicated the prospects of using the biofungicide to control numerous fungal and bacterial diseases during the potato growing season and storage of tubers. The crop rotation field experiment was carried out in 2017–2020 at the Experimental Station of the IEEP - BRANCH OF FSAC VIM near Saint-Petersburg (59°65 N and 30°38 E). The soil of experimental plots is sod-podzolic light loamy. In the potatoes (variety Udacha) field, a 2-factor field experiment was established which studied:

- the action of biofungicide Kartofin;
- the effect of the compost.

The experiment was established on the plots with the size 61.6 m^2 each. The experiment had four replications.
Three doses of the compost were used which corresponded to different levels of the potato productivity. Potatoes were treated with biofungicide at the time of planting and by foliar spray during the growing season. The combined use of compost at a dose of 4 t ha^{-1} and biofungcide made it possible to achieve the yield of standard tubers of 27.3–28.2 t ha^{-1} with their low incidence of fungal diseases. The biological effectiveness of the biofungicide Kartofin in reducing the prevalence and development of a complex of fungal diseases (alternariosis, late blight, stem form of rhizoctoniosis) on potato plants of the Udacha variety reached 82.2–89.9%.

Key words: biofungicide, biological control, organic cultivation, plant diseases, plant nutrition, potatoes.

INTRODUCTION

In Russia, organic agriculture has a share of 0.2%, however, production and consumption of organic products are starting to develop actively. On January 1, 2020, Federal Law No. 280-FZ 'On Organic Products and Amendments to Certain Legislative Acts of the Russian Federation' entered into force. The new law will, for the first time, begin to regulate manufacturing, storage, transportation, labeling, and marketing of organic products. Potato is the third most important food crop, after rice and wheat, with a total production of more than 300 million metric tons, as established by the International Potato Center (2017) in Lima, Peru. The Russian Federation ranks third in the world in the production of potatoes, which is cultivated in all major regions of the country, including North-Western region.

Two major challenges need to be addressed for sustainable organic potato production: efficient controlling of the phytosanitary state of the agroecosystem and ensuring adequate mineral nutrition of potatoes. Both factors are limited by regulations that, on the one hand, prohibit the use of artificial fertilizers, especially nitrogen fertilizers, and, on the other hand, most synthetic pesticides. In this regard, the selection of effective and safe microbiological pesticides to reduce the population density of phytopathogenic species is of particular importance.

However, organic management focuses on managing the environment, creating healthy soil and choosing more resilient plant varieties, which allow plants to resist potential attacks from microorganisms. Due to the reduced or non-existing input of mineral fertilizers and pesticides, organic fields tend to enhance biodiversity compared to conventionally managed fields, thus positively contributing to this SDG (de Schaetzen, 2019). Organic farms generally have more plant diversity, greater faunal diversity (insects, soil fauna and microbes, birds) and often more habitat and landscape diversity (Reganold & Wachter, 2016). The main practices that contribute to disease control are long, balanced rotations, organic amendments and reduced tillage, all geared towards maintenance of the soil organic matter content and fertility (Van Bruggen et al., 2015; De Tulipa & De L.C., 2019).

We selected and used a new polyfunctional biofungicide Kartofin, SC (suspension concentrate) to optimize the phytosanitary state of agroecosystem when growing organic potatoes. The producer strain of *Bacillus subtilis*-I5-12/23 was selected as a result of extensive screening of antagonist microbial strains that are part of the State Collection of Microorganisms Pathogenic for Plants and their Pests of the All-Russian Research Institute of Plant Protection. On the basis of this strain, the biofungicide Kartofin, SC was developed. The collection was registered at the World Federation for Culture Collections, World Data Center for Microorganisms (WFCC WDCM, Japan) under No. 760 on January 28, 1998.

Preliminary, studies were carried out in a series of model, vegetation and field experiments with Kartofin, SC. The results of studies indicated the prospects of using the biofungicide Kartofin to control numerous fungal and bacterial diseases during the potato growing season and storage of tubers. The indicators of biological and economic efficiency (Guidelines…, 2009) were used to assess the effect of the biofungicide on reducing the development of potato infection with fungal and bacterial diseases. The biological effectiveness of biofungicide Kartofin in relation to the prevalence and development of diseases was 50–100%, the economic efficiency of the use of biofungicide increased by 18.4–22.5% (Novikova et al., 2019; Titova et al., 2019).

Previously, Swedish scientists and Estonian scientists conducted field experiments and underlined that soil fertility had a strong influence on the productivity and quality of organic potatoes. So, organic potatoes needed to be cultivated on healthy soils with sufficient humus content and compost must be used to ensure that they have sufficient mineral nutrition. Our preliminary studies indicated that fairly high yields of organic potatoes may be obtained under the conditions of the Leningrad region using compost produced on the basis of chicken manure in an aerobic biofermenter (Popov et al., 2018; Minin et al., 2020).

In organic farming, the construction of the healthiest plant, provided with sufficient nutrients and favorable microbiota, is of particular importance. This contributes to an increase in plant disease resistance and protection from phytopathogenic microorganisms. At the same time, a favorable microbiota promotes the supply of more nutrients to the plants, which, in turn, promotes more active plant growth.

The considered model of organic potato predicts its development, using the emerging weather characteristics, forming favorable soil conditions and regulating the composition of its microecosystems by introducing biological products based on microbes-antagonists of phytopathogens.

At this stage, we use statistical models, on the basis of which a simulation model will be developed in the future.

MATERIALS AND METHODS

The field experiment was carried out in 2017–2020 at the Experimental Station of the Institute for Engineering and Environmental Problems in Agricultural Production (IEEP) - branch of FSAC VIM near Pavlovsk town, Saint-Petersburg (59°65 N and 30°38 E). The studies were carried out in the field organic crop rotation established in 2016. The crop rotation included the following crops: potato; red beat; barley with complementary seeding of clover and timothy; the 1st year perennial grass and legumes; the 2nd year perennial grasses; the 3rd year grass - after it's plowing in, the winter rye will be seeded for green manure. According to the Russian classification, the soil of experimental plots was sod-podzolic light loamy gleyic on residual carbonate moraine loam. It had a weak acidic reaction (pH = 6.5–6.6), high organic matter content (5.6%), and medium to high levels of available P and K.

In the field with potatoes (variety Udacha), a two-factor field experiment was established which studied the effect of the following factors:

- the action of biofungicide Kartofin, SC;
- the effect of the compost which provided the mineral nutrition of potatoes.

Experimental batches of biological fungicide Kartofin, SC based on *Bacillus subtilis* strain I-5 12/23 were developed for field trials. Producer strain is certified, deposited and maintained at the State Collection of Microorganisms Pathogenic for Plants and Their Pests at FSBSI VIZR (No. 760 on January 28, 1998 WFCC WDCM, Japan). Biological fungicide Kartofin, SC (titer not less than 5×10^{10} CFU mL^{-1}) is intended to protect agricultural crops from fungal and bacterial diseases during vegetation and storage of crops. The active biologic's ingredients are cells (spores) and a complex of *B. subtilis* strain I-5 12/23 metabolites; preparative formulation is the suspension concentrate (SC).

The biological fungicide was developed in accordance with the approved in the FSBSI VIZR regulations, specifications and toxicological passport. The biofungicide's experimental batches were prepared by the submerged cultivation method in the Microbiocontrol Laboratory FSBSI VIZR. The titer of the producer strain was determined by the serial dilutions' method with inoculation of the 8^{th} and 9^{th} dilutions on Dried nutrient agar: pancreatic sprat hydrolysate - 15 g L^{-1}, NaCl - 4.59 g L^{-1}, microbiological agar - 20 g L^{-1}, pH = 7.2 (Microgen Co. Ltd., Russia). The inoculum of the producer strain was preliminarily cultured in test tubes on the abovementioned oblique nutrient medium. The submerged cultivation was carried out in shaking flasks with 750 mL volume, containing 100 mL of nutrient medium: molasses - 15 g L^{-1}, corn extract - 30 g L^{-1}, pH = 7.8 (Research and Production Association 'ALTERNATIVE', Russia). Liquid phase inoculum of producer strain was grown at 27–28 °C for 3 days with aeration (180 rpm, New Brunswick™ Innova® 44 Shaker Incubator, Eppendorf, Germany). A sample was taken and microscoped to control the bacterial growth stage and the presence of foreign microbiota every day.

The fermentation process was stopped after 85–90 % spores producing in the culture liquid (CL). The CL was separated for 10 min at 3,000 rpm in the centrifuge OS-6MC (Dastan Inc., Kyrgyzstan) and then 0.2 % potassium sorbate was added to the spore suspension concentrate put into 1,000 mL wide-mouth bottles, PE-LD (VITLAB GmbH, Germany). The initial culture fluid titer was 5.2×10^{10} CFU mL^{-1}, the finished biologics Kartofin, SC titer was 8.2×10^{10} CFU mL^{-1}.

The studies were carried out with the compost produced in the Laboratory of organic waste bioconversion of IEEP - BRANCH OF FSAC VIM by the aerobic fermentation of the bedding poultry manure supplied by a poultry farm in the Leningrad Region (Briukhanov et al., 2017). It characterized by a high dry matter content (nearly 40%) and a high content of nitrogen and phosphorus (Table 1). Two compost doses were used; they were calculated by a sequentially doubling amount of nitrogen: 80 and 160 kg N ha^{-1} providing medium and high levels of potato productivity. By weight, this corresponded to application of 4.3 t ha^{-1} and 8.6 t ha^{-1} of compost. The compost was applied before ridging, followed by embedding the fertilizer by disking.

Table 1. The averaged characteristics of the compost

Parameter	Designation	Units	Value
Moisture content	MC	%	62.2
H^+	pH	-	8.5
N_{total} content	N_{total}	mg kg^{-1}	4,970.0
NH_4^+ content	NH_4^+	mg kg^{-1}	2,770.0
NO_3^- content	NO_3^-	mg kg^{-1}	890.0
K^+ content	K^+	mg kg^{-1}	2,890.0
P_{total} content	P_{total}	mg kg^{-1}	2,690.0
Ash content		%	19.2

The potato variety Udacha was cultivated. This potato variety is a new one, relatively resistant to late blight, perfectly adapts to various soils and weather conditions, it characterized by good taste and smoothness of the tuber surface (Shabanov, 2016). Potatoes were obtained from the seed farm (super elite and elite classes). Potatoes were treated with the biofungicide at the time of planting and by foliar spray during the growing season. For this purpose, a specially designed sprayer was installed on the planter and on the cultivator. Inter-row cultivation was carried out regularly, starting from the second week after planting, using an experimental specimen of a row-crop cultivator of an original design that provides deep loosing of inter rows. Weed vegetation was removed mechanically using small rotary harrow BRU-0.7 harrows mounted on the cultivator.

A plot size was 5.6×11 m = 61.6 m^2, with four replications in a complete randomized design. Potato planting in 2020 was carried out on May, 27, harvesting on September, 4.

All the results obtained in the experiment are placed in a database (Minin et al., 2020), which allows statistical processing of all research results using the Program STATISTICA 10. Mean comparison of data was made by using least significant difference (*LSD*) test at 5% error probability.

Observations of the growth rate of biomass (by the phases of plant development), phytosanitary conditions and soil properties were carried out regularly. Soil samples were taken from the arable horizon (0–25 cm) four times per season. Analytical studies were performed at the FSBSI VIZR Laboratory and the IEEP - BRANCH OF FSAC VIM Chemical Analytical Laboratory in accordance with the relevant GOSTs. Determination of nitrates and ammonium in soil was carried out using the ion metric method, GOST 26951-86.

RESULTS AND DISCUSSION

The sum of active temperatures (above 10° C) during the growing season (May - August) in the four years under consideration (2017–2020), varied from 1,625.5 to 2153.6. The average yield of the total potato biomass by variants varied from 17.4 to 40.8 t ha^{-1} at the same time.

The weather conditions presented in Table 2. It should be noted that if the sum of active temperatures characterizes the thermal and light regimes, then the hydrothermal coefficient (HTC) is a complex indicator reflecting both thermal and water regimes of the agroecosystem. The presented data indicate significant differences in the considered indicators between years.

Table 2. The sum of active temperatures and the hydrothermal coefficient (HTC) during the growing seasons of 2017–2020

Year	The sum of active temperatures			Hydrothermal Coefficient (HTC)			
	May - June	May - August	June - August	May	June	July	August
2017	573	1,626	1,431	0.45	1.69	2.48	2.83
2018	912	2,154	1,719	0.64	1.02	2.85	2.02
2019	901	1,941	1,600	2.11	1.42	3.52	1.80
2020	778	1,899	1,697	0.59	2.25	3.41	3.80

The correlation matrix, combining the results for all years and all indicators, indicates that the biological yield of potatoes had a closer relationship with the studied factors than the yield of standard products. Therefore, further we give more dependences linking biological production with the studied factors. However, there is a close relationship between biological yields and standard yields (Y), approximated by the equation:

$$Y = -4{,}105 + 0.794\ B1 + 0.003\ B2 \qquad R = 0.956$$

where B1 – total biological yield of potatoes; B2 – Sum of active temperature of May - August.

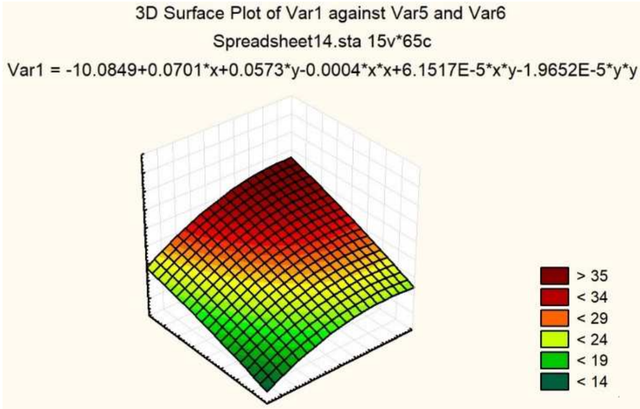

Figure 1. Dependence of the biological yield of potatoes (Var 1) on the compost dose (Var 5) and the sum of active temperatures in May - June (Var 6). $R = 0.800$.

We are interested in the relationship between yield of potatoes or proportion of diseased plants and the doses of compost and indicators of weather conditions. For this reason we use the software Program STATISTICA 10 and analysed our data with Multiple Regressions. We tried linear and quadratic dependencies and chosed those equations that had a higher correlation coefficient. Fig. 1 shows the dependence of the biological yield of potato tubers on the level of nitrogen applied with compost and the sum of active temperatures in May - June. Obviously, the presented quadratic function demonstrates non-linear relationships. The compost dose becomes more and more effective as the sum of active temperatures increases in May - June. In addition, it is important to note the great role of the temperature regime at the very beginning of potato development on its final yield. The Fig. 2 demonstrates the dependence of the share of potato tubers on the sum of active temperatures in May - June, at the very beginning of potato plant development, and moisture content in July.

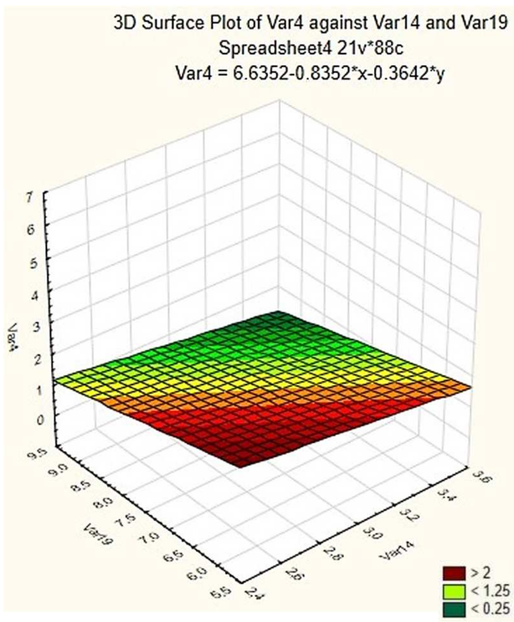

Figure 2. Dependence of the proportion of potatoes tubers damaged by diseases in the total yield (%) Var. 4 on the sum of active temperatures for May - June (Var. 7) and HTC for July (Var. 15) for 2017–2020. $R = 0.629$.

Influence of the compost and the biofungicide Kartofin, SC on potato yield during the growing seasons of 2017–2020 presented in Table 3. The use of the compost in the studied doses or Kartofin, SC (separately) led to an increase in yield by 1.2–1.3 times compared to the control without the use of fertilizer and biofungicide (Table 3).

Table 3. Influence of the compost and the biofungicide Kartofin, SC on potato yield during the growing seasons of 2017–2020

Variants Compost (kg of N ha^{-1})	Biofungicide	Years of investigation 2017	2018	2019	2020	The average yield for 2018–2020 years
	Total Biological Yield, t ha^{-1}					
0	-	-	21.3	23.3	20.8	21.0
80	-	-	29.6	30.9	25.0	28.0
0	Kartofin, SC	17.5	27.7	34.6	23.1	28.5
80	Kartofin, SC	23.0	32.9	35.7	28.5	32.4
160	Kartofin, SC	20.7	33.7	43.0	34.2	37.0
$LSD_{0.95}$		1.6	3.5	5.4	3.4	
	Yield of Standard Tubers, t ha^{-1}					
0	-	-	17.1	22.8	20.3	20.1
80	-	-	21.3	30.5	25.9	25.9
0	Kartofin, SC	14.0	24.2	29.5	22.5	25.4
80	Kartofin, SC	18.7	27.6	32.0	26.9	28.8
160	Kartofin, SC	17.3	25.8	42.2	31.8	33.3
$LSD_{0.95}$		1.6	1.8	4.0	2.1	

The combined use of compost and Kartofin, SC provided an increase in the yield of the total mass of tubers and standard potato tubers by 1.5 times. It was the synergistic effect of these biological products, which persisted throughout the entire research period for organic potato cultivation during the growing seasons of 2017–2020 (Table 3). Such a steady trend of obtaining the organic potatoes' increased yields in four years is due to the phytosanitary situation improvement in planting potatoes when using the biofungicide Kartofin, SC as well as providing nutrition to potato plants through the use of organic fertilizers. An improvement in the habitus and development of plants, a decrease in the diseases' development of the aerial part during each growing season and, as a consequence, a decrease in the tubers damage by phytopathogens were observed annually. More detailed studies of the potato diseases controlling by the biofungicide during the growing season were carried out in 2020.

Table 4. The influence of the biofungicide Kartofin SC and the compost on the spread of fungal diseases on potatoes of the variety Udacha in the growing season 2020

Variants	Prevalence / development of fungal diseases Development phase of aerial potato plants part, % Bud-formation	Blooming	Closing the rows	Before potato herbage cutting	On tubers, %
Control 1 (without biofungicide)	2.3/0.6	29.4/14.2	22.6/11.4	48.1/33.1	100/49.1
Control 2 (without biofungicide) + Compost 80 kg of N ha^{-1}	0	16.5/7.3	11.5/6.1	36.2/24.6	100/45.7
Kartofin, SC	0	3.9/1.6	4.1/2.1	20.6/11.2	92.3/44.6
Kartofin, SC + Compost 80 kg of N ha^{-1}	0	3.1/1.1	3.2/1.7	30.2/18.6	97.9/38.0

Note: disease prevalence / disease development.

The use of the biofungicide Kartofin, SC had a significant effect on the phytosanitary state of the organic potatoes' agroecosystem by the 4th year of research – the growing season 2020 (Tables 3, 4).

The prevalence of fungal diseases on potato plants decreased by 2 times in comparison with the control both without compost and with its use in the studied doses (Table 4). The best effect of using biofungicide was revealed in a threefold decrease in the development of fungal diseases of potato plants (Table 4). A 1.3-fold persistent decrease in the development of fungal diseases on tubers was also observed (Table 4).

The biological effectiveness of the biofungicide Kartofin, SC and the compost, calculated for the 4th year of research, is presented in Table 5. The data indicates the high efficiency of the biofungicide in organic growing of potatoes: the prevalence and development of fungal diseases decreased by more than 3 times as in plants by the end of the growing season, and on tubers (Table 4). The efficiency of Kartofin, SC in reducing the prevalence and development of fungal diseases during the growing season and during the formation of tubers was more than 80%. The combination of compost and biofungicide reduced the prevalence and development of fungal diseases on 85–92%, due to the synergistic effect (Table 5).

Table 5. Biological efficiency of the biofungicide Kartofin, SC and the compost on Udacha variety potatoes in the growing season 2020

Variants	Biological effectiveness in reducing the prevalence / development of fungal diseases				
	Development phase of aerial potato plants part, %				On tubers, %
	Bud-formation	Blooming	Closing the rows	Before potato herbage cutting	
Compost 80 kg of N ha^{-1} (without biofungicide)	100/100	43.8/48.8	49.3/46.1	24.4/25.6	0/6.8
Kartofin SC	100/100	86.8/88.7	82.2/81.2	57.1/66.1	7.7/8.9
Kartofin SC + Compost 80 kg of N ha^{-1}	100/100	89.9/92.1	86.1/84.8	37.1/43.8	2.1/22.3

Note: disease prevalence / disease development.

Thus, the biological effectiveness of the biological product Kartofin, SC in reducing the prevalence of a complex of fungal diseases (alternariosis, late blight, stem form of rhizoctoniosis) on potato plants of the Udacha variety reached 82.2–89.9%. The development of fungal diseases on potatoes growing on the plots where the biofungicide Kartofin SC was applied was significantly (3–9 times) lower than in the control plots without biofungicide application, during the entire observation period - 1.6–11.2% and 14.2–33.1%, respectively. The biological effectiveness in reducing the development of a complex of fungal diseases was estimated in the range of 81.2–92.1%. A synergistic effect was observed with the combined use of the studied doses of compost and biofungicide Kartofin, SC, leading to a 2–3-fold increase in efficiency.

It was shown that the biofungicide Kartofin SC reduced the infection of tubers in comparison with the control by 5–11%, providing 9–22% efficiency of its application. The biological yield of tubers of the Udacha variety on the control plots without compost application was 20.8 t ha^{-1}, with the application of compost - 25.1 t ha^{-1}. In variants with the use of a biofungicide and in combination with compost, this figure reached 23.1–25.8 t ha^{-1}. Thus, the use of organic fertilizers and biofungicide provided an increase in the yield of tubers of the Udacha variety by a factor of 1.5 compared to the control without the application of compost and a biofungicide. The yield of standard tubers increased from 20.2 t ha^{-1} in the control to 24.1 and 25.1 t ha^{-1} with the addition of compost and biofungicide, respectively.

CONCLUSIONS

1. As a result of the analysis of information for four years, a particular value of the sum of active temperatures in May - June was established to ensure a high yield of organic potatoes and reduce the incidence of tuber diseases.
2. The efficiency of the selected version of the technology for the production of organic potatoes has been confirmed. The combined use of compost at a dose of 4 t ha^{-1} and the biofungicide Kartofin, SC made it possible to achieve the yield of standard tubers of 27.3–28.2 t ha^{-1} with their low incidence of fungal diseases.
3. The results obtained make it possible to recommend the inclusion of the biofungicide Kartofin SC in the developed technology for the cultivation of organic potatoes. The biofungicide Kartofin, SC had a significant impact on the yield of potatoes of the Udacha variety and the phytosanitary state of the agrobiocenosis of organic potatoes, reducing the prevalence and development of a complex of fungal diseases on vegetative plants and tubers. The biological effectiveness in reducing the development of a complex of fungal diseases was estimated in the range of 81.2–92.1%. A synergistic effect was observed with the combined use of the studied doses of compost and biofungicide Kartofin, SC, leading to a 2–3-fold increase in efficiency.

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