


## Changes in the seed and soil micobiota caused by seed treatment with chemical and biological agents

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### ABSTRACT

This study investigates the impact of chemical and biological treatments on the mycobiota of winter wheat seeds and the fungal composition of the soil. Conducted in 2023–2024 using the winter wheat variety Aliot, the research aimed to determine how different seed treatments affect the microbial populations and the development of wheat seedlings. The experiment was carried out in Sumy, Ukraine, at the Educational Research and Production complex of Sumy National Agrarian University. Chemical treatments tested included Tebuzan Ultra F.C.S., Celest Top 312.5 FS TH, Maxim 025 FS TH, and Record F.C.S., while biological agents included Azotobacterin-K BI, ECOSTERN Trichoderma CS, Bacillus megaterium, and others. Seeds were treated and their mycobiota were analyzed using biological methods to assess changes in fungal and bacterial populations. Additionally, treated seeds were sown to study the effects on the soil microbiota. The results showed that chemical treatments effectively suppressed several fungal species, particularly *Alternaria tenuissima*, and led to an increase in bacterial colonies within the seeds. However, they also reduced microbial diversity in the soil, which in some cases negatively impacted seedling development. In contrast, biological treatments, while less effective at completely suppressing fungal pathogens, enriched the seed microbiota, particularly increasing *Aspergillus oryzae* populations, and promoted the growth of beneficial soil microorganisms. Biological agents such as Azotobacterin-K BI and ECOSTERN Trichoderma CS significantly improved seedling length, reaching up to 13.59 cm, compared to chemical treatments which sometimes reduced seedling growth. Overall, the study highlights that while chemical treatments provide immediate protection against pathogens, biological treatments offer long-term benefits by enhancing microbial diversity and promoting healthier plant growth. These findings support the growing trend toward sustainable agriculture, where biological agents can be integrated to reduce chemical inputs and improve environmental safety without compromising yield.

**Keywords:** Mycobiota, winter wheat, biological agents, chemical fungicides, soil micobiota.

### INTRODUCTION

Winter wheat is one of the most important grain crops in the world, providing a significant portion of the global food supply. However, it is susceptible to various diseases that can cause substantial yield losses (Rebouh, et al. 2022). Seed treatment is one of the key agronomic measures aimed at protecting winter wheat from

a wide range of pathogens that can significantly reduce its productivity (Poole and Arnau-din 2014). The use of chemical and biological agents for seed treatment remains a relevant issue in modern agriculture, as these methods provide varying levels of effectiveness depending on the type of pathogen and growing conditions (Albajes, et al. 2000). In recent decades, there has been increasing attention to biological

methods of plant protection, driven by their environmental safety and lack of negative impact on the environment compared to chemical fungicides, which can have harmful effects on human health and the ecosystem (Sharma, et al. 2015).

It is important to note that the effectiveness of seed treatment depends on several factors, such as the composition of pathogens present in the soil, weather conditions, and the chosen treatment method (Klipakova and Bilousova 2018). In the context of increasing demand for sustainable agricultural practices, a comparative analysis of the effectiveness of chemical and biological agents for seed treatment becomes particularly significant.

Chemical and biological seed treatments are widely used in agronomy for seed protection. Chemical seed treatments effectively eliminate a broad range of microorganisms, providing fast and reliable protection (Markovska, et al. 2020). However, their use may have negative environmental consequences, including soil and water contamination, as well as the development of pest resistance to active substances (Lykogianni, et al. 2021). The use of pesticides can lead to the accumulation of toxic substances in soil and water, posing a threat to the health of humans and animals. Residual pesticides, which remain in products, are particularly harmful and can negatively affect the respiratory, nervous, and endocrine systems (Trokhymenko, et al. 2022). On the other hand, biological seed treatments are more environmentally friendly, as they are based on natural microorganisms or their metabolites, reducing the risk of negative impact on ecosystems (Nega 2014). They also contribute to increased biodiversity and improved soil conditions (Lemanceau, et al. 2014). At the same time, their effectiveness can be less stable and dependent on environmental conditions, requiring careful monitoring and potentially limiting their use in certain cases. Thus, the choice between chemical and biological seed treatments depends on specific agronomic tasks and environmental requirements (Sharma et al. 2015).

Modern seed treatments are rapidly evolving, with new innovations continuously introduced to enhance plant protection efficacy. For example, in combating fungi of the genus *Fusarium*, which cause many wheat diseases, *Bacillus velezensis* RC 218 can be highly effective in biological control, reducing *Fusarium graminearum* Schwabe infection and the accumulation of toxic

trichothecenes (Cantoro, et al. 2021). To suppress the growth of *Rhizoctonia cerealis* Hoeven, the main pathogen causing wheat diseases, *Bacillus subtilis* XZ18-3 is used, demonstrating high efficacy in pathogen control, achieving 88.28% success in experiments (Yi, et al. 2022).

Modern technologies allow the creation of innovative products that more effectively target specific threats. For the effective control of septoria, brown rust, fusarium, and alternaria, an innovative product called ADEPIDYN (*pydiflumetofen*) has been developed. It is the first member of the class of succinate dehydrogenase inhibitors (SDHI) (Stierli, et al. 2021).

The development of new disease control methods significantly improves wheat protection, contributing to increased yields and reducing losses during cultivation. To improve effectiveness significantly, scientists recommend design systems that combine the use of various treatments and incorporate modern advances in biological production across different crop rotations and winter wheat protection systems. This approach can substantially boost the yield of this crop, expand the applicability of these technologies, and reduce the need for pesticide use (Chugrii 2020; Zayets, et al. 2020).

In year 2024 Ukraine has 38 certified biofungicides that meet the requirements of organic production (according to the certification body “Organic Standard”), and more than 150 biopreparations (according to the State Register of Pesticides and Agrochemicals Permitted for Use in Ukraine). This reflects a growing trend toward the adoption of environmentally safe plant protection products in agricultural practices. At the same time, the number of registered chemical fungicides significantly exceeds their biological counterparts, with over 800 products available (according to the State Register of Pesticides and Agrochemicals Permitted for Use in Ukraine). This situation indicates that chemical agents remain dominant in Ukrainian agriculture, particularly for large agricultural enterprises. However, with the increasing interest in organic farming and global demands for environmental safety, further development of the biopesticide market is expected, which will help reduce the impact of chemical agents on the environment and human health (Havran, et al. 2024).

The aim of the study was to determine the impact of chemical and biological treatments on the mycobiota of winter wheat seeds and the fungal

complex of the soil mixture, considering the development of seedlings.

## MATERIALS AND METHODS OF RESEARCH

The research was conducted during 2023–2024, using the winter wheat variety *Aliot*. The study site was located in Ukraine, Sumy region, in the city of Sumy, at the territory of the Educational, Research and Production complex of Sumy National Agrarian University.

Initially, the seeds were treated with preparations of different origins and placed on agar medium to study changes in the mycobiota under the influence of their active ingredients. Afterward, the treated seeds were sown in soil to observe changes in the fungal complex of the soil.

The analysis of seed mycobiota was carried out using a biological method with potato dextrose agar (PDA) to determine internal infections of the seeds (Zhukova, et al. 2023). The soil samples were analyzed using the following media: bacteria were cultured on meat-peptone agar (MPA), while fungi were cultured on Czapek-Dox medium with glucose. From each sample, 10 grams of soil were taken using a sterile spatula. The dilution method was used to determine the number of microorganisms in the soil samples, after which the fungal species were identified (Biliavska, et al. 2023).

The seeds were grown on a soil-peat substrate, which was evenly distributed in 500 ml plastic containers. For each experimental sample, 50 seeds were sown. The growing process was carried out under conditions of a full light day, with temperatures maintained between 18–20 °C.

The representatives of the mycobiota were identified based on the characteristics of their colony structure on the medium and the morphology of conidial spore formation (Watanabe 2002; Woudenberg, et al. 2013).

The chemical seed treatments used in the experiment included the following: 1) Tebuzan Ultra, F.C.S. (flowable concentrate suspension) – active ingredient: tebuconazole, 120 g/L. 2) Record, F.C.S. – active ingredients: carboxin, 170 g/L, and thiram, 170 g/L. 3) Celest Top 312.5, FS, TH (FS - Flowable Concentrate for Seed Treatment; TH - concentrate suspension) – active ingredients: fludioxonil, 25 g/L; difenoconazole, 25 g/L; thiamethoxam, 262.5 g/L. 4) Maxim 025, FS, TH – active ingredient: fludioxonil, 25 g/L.

The biological preparations used in the experiment included: 1) Azotobacterin-K, BI (bioinoculant) – contains strains of *Azotobacter chroococcum* and *Azotobacter vinelandii*, bioactive metabolites, microelements, and nutrients, with a live cell titer of  $5\text{--}7 \times 10^8$  cells/ml. Suitable for seed treatment, seedling care, and plant spraying. 2) Phytovit, BE (biological extract) – includes supernatant from the culture fluid and ethanol extract of biomass in a 4:1 ratio, derived from *Streptomyces netropsis* IMV Ac-5025. The preparation is aimed at stimulating biological processes, enhancing immunity, and increasing resistance to stress factors. It may also have antimicrobial and immunomodulatory properties due to its composition. 3) Violar, BE – contains supernatant from the culture fluid and ethanol extract of biomass in a 4:1 ratio, obtained from *Streptomyces violaceus* IMV Ac-5027. This preparation uses biologically active substances to influence various organisms and has a broad spectrum of biological activity. 4) *Bacillus megaterium* de Bary – a live culture provided by IMV D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Kyiv. 5) ECOSTERN Trichoderma, CS (concentrated suspension) – includes spores and mycelium of antagonistic fungi of the genus *Trichoderma*, with a concentration of viable effective microorganisms not less than  $1 \times 10^7$  CFU/cm<sup>3</sup>. The metabolic products of these microorganisms include antibiotic substances that effectively suppress the growth of many pathogenic agents. 6) AVERCORM-H, CB (complex biological preparation) – includes supernatant of the culture fluid and Avercom (biomass extract of *Streptomyces avermitilis* IMV Ac-5015 containing 100 mg/L avermectin) in a 1:1 ratio, with the addition of Chitosan at a concentration of 0.01 mM as an elicitor. 7) Sporozin, S (suspension) – *Pseudomonas aureofaciens* Mb-24 (IMV B-7559), *Pseudomonas aureofaciens* Mb-17 (IMB B-7558), *Bacillus subtilis* BT-7 (IMB B-7349) with a total titer of not less than  $3 \times 10^8$  CFU/ml. 8) Complex – a combination of four preparations: Phytovit, BE + Violar, BE + AVERCORM-H, CB + Sporozin, S.

## RESULTS

Research on the mycobiota of wheat seeds during 2022–2023 showed the dominance of *Alternaria tenuissima* Wiltshire, as well as the presence of a representative of the genus *Fusarium*

— *Fusarium oxysporum* Schlecht. Isolates of this species were distinguished by pink colony coloration (Fig. 1).

The study of the effects of treatments on seed mycobiota showed that *A. tenuissima* dominated in the control group, where the widest spectrum of fungi was observed. Chemical treatments proved effective against the most abundant *Alternaria* fungus and triggered the emergence of bacteria within the seeds (Table 1). The chemical treatments Celest Top 312.5, FS, TH and Maxim 025, FS, TH showed a reduction in the number of *Mucor* spp. and *Aspergillus oryzae* Cohn colonies. However, in the variant with Maxim 025, FS, TH, *F. oxysporum* appeared, though in significantly smaller quantities compared to the control, while *Cladosporium* spp.,

which was not observed in the control, appeared with Celest Top 312.5, FS, TH. It should also be noted that the number of bacterial colonies increased significantly with both Celest Top 312.5, FS, TH and Maxim 025, FS, TH. The chemical treatment Tebuzan Ultra, F.C.S. showed an increase in the number of *Mucor* spp., although it suppressed the development of all other fungi. The use of *Record*, F.C.S. reduced the development of *Mucor* spp., but increased the quantity of *A. oryzae*. The most effective treatments for reducing fungal mycobiota numbers were Tebuzan Ultra, F.C.S. and *Record*, F.C.S.

Biological treatments also significantly reduced the population of the dominant species, particularly Azotobacterin-K, BI, but increased the isolation of *A. oryzae*. ECOSTERN Trichoderma,

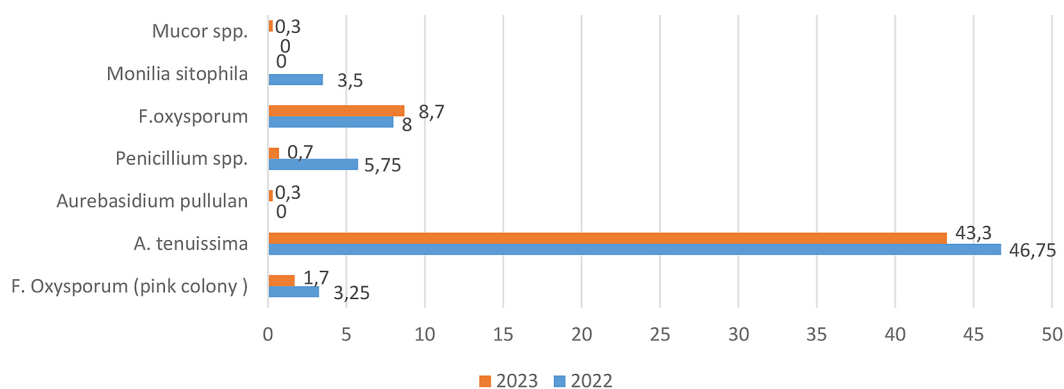


Figure 1. Mycobiota of winter wheat seeds in the Northeastern Forest-Steppe of Ukraine (2022–2023) (percentage of isolation, %)

Table 1. Effect of seed treatment on its mycobiota (Aliot variety, 2023 harvest, % isolation)

View	Name of the product	Mucor sp.	Bacteria	Aspergillus oryzae	Cladosporium sp.	A. tenuissima	A. infectoria	Penicillium sp.	F. oxysporum	F. Oxysporum (pink colony)	Arthrinium arundinis	Aurebasidium pullulan	Other types
Chemical	Tebuzan Ultra, F.C.S.	12*	5	–	–	–	–	–	–	–	–	–	1*
	Record, F.C.S.	5*	3	18*	–	–	–	1*	–	–	–	–	–
	Celest Top 312.5, FS, TH	4*	59	4	14	–	–	–	–	–	–	–	–
	Maxim 025, FS, TH	1*	31	3	–	–	–	–	–	2	–	–	2*
Biological	Azotobacterin-K, BI	8*	–	37*	–	2*	–	–	–	–	–	–	–
	Phytovit, BE	15*	–	24*	–	17*	–	–	–	–	–	–	–
	Violar, BE	–	–	17*	1	21*	2	–	–	–	3	–	–
	B. megaterium	1*	–	74*	–	6*	–	–	–	–	–	–	–
	Complex	10	–	30*	–	18*	–	–	–	1	–	–	–
	ECOSTERN Trichoderma, CS	3*	–	12*	–	12*	–	–	–	–	–	–	–
Control	–	11	–	4	–	53	–	4	5	–	–	2	8

Note: \*there is a significant difference between the control and the variant according to Fisher’s test (F05)

CS and *B. megaterium* notably reduced the quantity of *Mucor* sp. and *A. tenuissima*. However, in the variant with *B. megaterium*, the number of *A. oryzae* colonies was the highest recorded. The treatment Violar, BE suppressed the development of *Mucor* sp., reducing colony numbers to zero, and diminished the growth of all other species compared to the control. Nevertheless, small amounts of *Cladosporium* spp., *Alternaria infectoria* Simmons, and *Arthrimum arundinis* Eisenmenger colonies appeared, which were not observed in the control. In the variants with Azotobacterin-K, BI, Complex, and AVERCORM-H, CB, a significant reduction in *A. tenuissima* was noted. In the Complex sample, *F. oxysporum* also appeared. In the Phytovit, BE variant, an increase in *Mucor* spp. and a significant decrease in *A. tenuissima* were observed. The most effective treatments in reducing fungal numbers in the seeds were ECOSTERN Trichoderma, CS and Azotobacterin-K, BI.

Comparing chemical and biological treatments, several key differences can be highlighted: chemical treatments completely suppressed *A. tenuissima*, whereas the use of biological treatments only reduced its quantity. Chemical treatments decreased the number of *A. oryzae* colonies (except in the Record, F.C.S sample), while biological treatments significantly increased the number of *A. oryzae* colonies. In most cases, both chemical and biological treatments suppressed the growth of *Mucor* spp., except for the Tebuzan Ultra, F.C.S. (chemical) and Phytovit, BE (biological) samples, which showed an increase in the number of colonies of this species. It is important to note that the application of chemical treatments led to the appearance of bacterial colonies, which were not observed in the samples treated with biological treatments or in the control group.

While studying the mycobiota of the soil mixture, two fungal species were identified: *Penicillium cheresanum* Biourge and *Trichoderma* sp. The effect of seed treatment on changes in fungal and bacterial populations was investigated (Table 2).

The use of chemical seed treatments led to a reduction in the overall number of fungi compared to the control. The lowest number of fungi was recorded after applying Celest Top 312.5, FS, TH –  $6.2 \times 10^5$  CFU/g. The reduction in fungal counts varied among the chemical treatments: Celest Top 312.5, FS, TH and Tebuzan Ultra, F.C.S. significantly reduced *P. Cheresanum* and *Trichoderma* sp., while Maxim 025, FS,

TH increased the number of the former species and decreased the latter. Record, F.C.S. significantly reduced only *Trichoderma* sp. Regarding bacteria, most chemical treatments also reduced their numbers, except for Record, F.C.S., which increased their count.

Biological treatments both decreased and increased the number of fungi in the soil mixture. The application of a mixture of biopreparations reduced the number of fungi but increased the number of bacteria. Only Phytovit, BE reduced the number of *P. Cheresanum*, while all other biopreparations effectively reduced *Trichoderma* sp., especially Azotobacterin-K, BI. The highest number of microorganisms was observed with the application of *B. megaterium*.

An analysis of seedling length showed that biological treatments increase seedling length compared to the control, with *B. megaterium* and Azotobacterin-K, BI having the most significant positive impact, increasing the length to 12.93 cm and 13.59 cm, respectively. The chemical treatment Record, F.C.S., also contributed to a significant increase in seedling length, reaching 11.92 cm. Meanwhile, some chemical treatments, such as Celest Top 312.5, FS, TH and Tebuzan Ultra, F.C.S, reduced seedling length to 10.53 mm and 8.67 mm, respectively.

Overall, the results show that different seed treatments for wheat significantly affect the composition and quantity of microorganisms in the soil. The impact of chemical treatments generally leads to a reduction in the number of microorganisms, while biological treatments promote their increase. The analysis of wheat seedling development shows that biopreparations have the most pronounced positive effect, whereas the impact of chemical treatments can range from positive to negative.

## DISCUSSION

The results of this study confirm the significant impact of both chemical and biological treatments on the seed mycobiota of wheat, the soil, and wheat seedling growth. The use of chemical treatments such as Celest Top 312.5, FS, TH and Maxim 025, FS, TH reduced the number of fungi like *Mucor* spp. and *A. oryzae*, but at the same time, promoted the emergence of new species, such as *Cladosporium* spp. This indicates a selective effect of chemical agents on different

**Table 2.** Effect of seed treatment on wheat seedling length and soil microbiota

№	Example	Length of seedlings	Number of microorganisms (CFU/g of dry soil)		
			Fungi on Czapek-Dox		Bacteria on MPA ( $\times 10^6$ )
			Species count ( $\times 10^5$ )	Total	
1	Control	10.72	<i>P. cheresanum</i> - 7.2	10.6	1.6
			<i>Trichoderma</i> sp. - 3.4		
2	Tebuzan Ultra, F.C.S.	8.72*	<i>P. cheresanum</i> - 5.9	6.2	0.6
			<i>Trichoderma</i> sp. - 0.3		
3	Record, F.C.S.	11.92*	<i>P. cheresanum</i> - 7.1	7.4	1.9
			<i>Trichoderma</i> sp. - 0.3		
4	Celest Top 312.5, FS, TH	10.56	<i>P. cheresanum</i> - 3.3	3.8	0.9
			<i>Trichoderma</i> sp. - 0.5		
5	Maxim 025, FS, TH	10.88	<i>P. cheresanum</i> - 9.7	10.2	1.3
			<i>Trichoderma</i> sp. - 0.5		
6	Azotobacterin-K, BI	13.59	<i>P. cheresanum</i> - 10.4	10.7	0.9
			<i>Trichoderma</i> sp. - 0.3		
7	Phytovit, BE	11.47	<i>P. cheresanum</i> - 5.8	7.3	2.6
			<i>Trichoderma</i> sp. - 1.5		
8	Violar, BE	12.73*	<i>P. cheresanum</i> - 8.6	9.2	2.1
			<i>Trichoderma</i> sp. - 0.6		
9	<i>B. megaterium</i>	12.93*	<i>P. cheresanum</i> - 12.5	13.5	5.9
			<i>Trichoderma</i> sp. - 1		
10	Complex	11.16	<i>P. cheresanum</i> - 9.5	9.9	2.3
			<i>Trichoderma</i> sp. - 0.4		
11	ECOSTERN Trichoderma, CS	12.52*	<i>P. cheresanum</i> - 7.4	8.0	3
			<i>Trichoderma</i> sp. - 0.6		
12	AVERCORM-H, CB	11.85*	<i>P. cheresanum</i> - 7.8	9.0	1.1
			<i>Trichoderma</i> sp. - 1.2		

**Note:** \* there is a significant difference between the control and the variant

species of microorganisms, which is supported by other studies (Abdel-Kader, Moubasher and Abdel-Hafez 1978). For example, difenoconazole and fludioxonil can effectively inhibit the development of certain fungal species while creating conditions favorable for the growth of others (Sayoko, et al. 2022; Franz, et al. 2020).

Biological treatments, particularly *B. megaterium* and ECOSTERN Trichoderma, CS demonstrated a significant effect in reducing the numbers of *Mucor* spp. and other pathogenic fungi. This is consistent with literature data highlighting the high effectiveness of microorganisms in the biological protection of plants (Ferreira and Musumeci 2021; Hernández-Castillo, et al. 2020).

Data analysis showed that in some cases, biological treatments such as *B. megaterium* contribute to an increase in the number of bacteria and fungi (*A. oryzae*). This may result from active competition between microorganisms for resources or

changes in the physicochemical properties of the soil under the influence of biopreparations, as supported by research findings (Yuexia, Wang and Li 2016; Koshila Ravi, et al. 2019).

A comparison of the effectiveness of chemical and biological treatments also showed that chemical treatments based on tebuconazole can suppress the development of most fungi, but in some cases, they promote an increase in the number of other species, such as *Mucor* spp (Garcia, Lee and Courtney 2023).

Biological treatments such as *B. megaterium* and Azotobacterin-K, BI show better results, not only reducing the number of pathogenic fungi but also stimulating wheat seedling growth, which is also confirmed by research. In their studies, Shokry Mohamed El-Grem et al. (2017) demonstrated that the bacterial isolate *B. megaterium* effectively suppresses the development of fungal pathogens like *Cochliobolus sativus* S. Ito &

Kurib., *Alternaria alternata* (Fr.) Keissl, and *F. graminearum*. Moreover, the use of this isolate led to an increase in plant shoot length: in the control group, this indicator was 13.96 cm, while in the variant with the isolate, it reached 19.81 cm (in vitro) (El-Gremi, Draz and Youssef 2017).

Overall, the results of this study are consistent with current scientific data, which emphasize the importance of biopreparations in plant protection and enhancing the productivity of agroecosystems. The use of such treatments in agriculture is becoming increasingly relevant due to their ability to provide disease resistance and promote plant growth without negative impacts on the ecosystem (Dayan, Cantrell and Duke 2009).

The novelty of this study lies in the comparison of the effects of chemical and biological treatments on the seed mycobiota of wheat and soil in the conditions of the Northeastern Forest-Steppe of Ukraine. The results demonstrate that biological treatments are promising for integrated farming, providing not only protection against pathogens but also improving plant growth.

## CONCLUSIONS

The seed microbiota of wheat underwent significant changes under the influence of various treatment methods, including chemical and biological agents. Chemical treatments effectively suppressed the growth of certain fungal species, such as *A. tenuissima*, but led to the emergence of bacterial colonies, which were not observed in control or biological samples. In contrast, biological agents enriched the microbiota, specifically increasing the number of *A. oryzae* colonies and stimulating the development of beneficial soil microflora. Chemical treatments reduced the number of microorganisms in the soil, which in some cases could negatively impact germination, whereas biological agents provided the most pronounced positive effect on plant growth and their microbiological condition.

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