

## The Effectiveness of a Biotechnological Method for Controlling the Content of Gossypol

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### Abstract

The results of studies devoted to the study of the occurrence of gossypol are presented; factors contributing to the formation of gossypol are listed. A new method for controlling the content of gossypol in cottonseed has been developed: it has been experimentally established that when cottonseed is soaked in a solution of water of cattle manure, lamb manure and poultry manure, and the catalyst, a decrease or increase in the content of gossypol is observed. When soaking cottonseed with residual fiber in the solution, the level of gossypol decreased to 0.063%. Experiments have shown that with a concentration of 21 kg of cattle manure, 21 kg of sheep manure and 13 kg of poultry manure, 25 g of urea and ammonium nitrate each in 14 liters of water, the process proceeds rationally towards a decrease in gossypol. The essence of the method for determining the quantitative content of gossypol consists in the extraction of free gossypol from the analyzed product with a mass fraction of moisture not exceeding 7% with anhydrous acetone and subsequent quantitative determination of gossypol by a photometric method according to existing standards. Due to the naturalness of the components of the solution, cottonseed oil obtained from cottonseed, processed by a biotechnological method, can be used as a healing one. Therefore, a new way of managing gossypol content is beneficial.

**Keywords:** gossypol, cotton, biotechnology, manure, solution.

### Introduction

Researchers have carried out numerous experiments to study gossypol. Gossypol is a product obtained from the processing of cottonseeds or from the roots of cotton (*Gossypium* sp.), Malvaceae family (Malvaceae). Fine-crystalline powder from light yellow to dark yellow in color with a greenish tinge. It gets dark in the light. Practically insoluble in water, slightly soluble in alcohol, [1].

As a result of a systematic study of cotton, more than 100 chemical compounds belonging to different classes of organic chemistry have been identified from its vegetative and generative organs: organic and fatty acids, phytosterones, triglycerides, polyphenols, carotenoids, High molecular hydrocarbons, alcohols and others. The study of the substances isolated from cotton has made it possible to establish the chemical structure and voyage of these complex compounds, and it is no less important to prove that cotton is an inexhaustible store of more than 1,200 minerals, necessary for the development of the chemical and pharmacological industry. Gossipole is found

in small quantities in leaves, stem bark, flaps of boxes, seed husks and cotton flowers.

The highest concentration of Gossipole is found in root bark (1.29-3.0 per cent) and seed kernels (0.2-2.03 percent), [2].

A new method was developed for determining the gossypol content in cotton oil using IR-Fourier spectroscopy with a pass cell NaCl... Due to its speed (about 2 min) and the ease of data processing, FTIR spectroscopy is a useful alternative to standard wet chemical methods for fast and routine determination of the gossypol in the process and / or quality control of cotton oil [3].

Two forms of the Gossippol have been found, free and bound. A related form is formed by covalent bonds between the gossip-pol and free epsilon-aminogroups of lysine and arginine via a darker or Mayar reaction, [4, 5, 6, 7, 8, 9].

It has been found that the gossypol affects male and female gametogenesis and contributes to

embryo lesions [9, 10]. Based on reviews of sources and their numerous experimental studies, scientists have come to the following conclusions: ingestion of the gossip-polo present in cottonseeds and products may contribute to clinical poisoning, Liver damage, male and female reproductive toxicity, and immunological disorders. Acute poisoning is currently not a major problem, but reproductive damage is causing serious economic damage to livestock and industry. Although male reproductive toxicity is well known, more research is needed to understand the damage to the female reproductive system caused by the gossipol. The immunotoxicity of gossypol is far from complete, but it affects animals by decreasing their resistance to infection and by reducing the effectiveness of vaccines. Extensive research is required to develop more effective and inexpensive technologies for reducing the toxicity of gossypol.

Gossypol is easily absorbed in the gastrointestinal tract, slowly excreted from the body. Etching symptoms (increased peristalsis, persistent diarrhea, frequent and painful urination, then cough, pulmonary edema, venous blood congestion) usually appear after a few days [11].

Removing gossypol from oil and meal presents certain difficulties. Despite the existence of several methods for removing gossypol described in the literature, there is still no radical way to completely extract it from oil and meal. One of the reasons for this is the insufficient knowledge of the chemical transformations of gossypol, [12]. It has been established that the use of biotechnology in the modification of cotton makes it possible to improve the production technology and reduce the use of chemical reagents that have a negative impact on the environment [13].

Biotechnology is the production of products and materials necessary for a person using living organisms, cultured cells and biological processes. In 1981, the European Federation of Biotechnology defined the integrated use of biochemistry, microbiology and chemical technology to achieve the technological application of the capabilities of microorganisms and cultured tissue cells. It is not easy to isolate the field of biotechnology because it coincides with several industries, such as the chemical and food industries, but biotechnology has also found widespread application in the textile industry, in particular in textile and waste management [14].

In the traditional process of adsorption purification of vegetable oils, the main technological factors are: temperature, stirring intensity (number), pressure, adsorbent quantity, etc. The quality indicator of the adsorption process is more often the degree of purification (bleaching) of vegetable oils, which in each case is determined according to the type of oil being treated, its use, etc. In the process of adsorption purification of cotton oil colorants (gossipol, chlorophyll and their derivatives), residues of soap, hydrocarbons, pesticides, defoliants, etc., are mostly removed. [15].

Cotton oils derived from III-IV seeds and non-standard seed varieties, as well as from technological deviations at the extraction, extraction and especially distillation stage, are referred to as "hard to refine" oils.

These oils are characterized by a high content of free fatty acids, phospholipids, non-flammable lipids and colorants: chlorophylls, gossipole and its modified and derived forms, or a predominant content of colorants with moderate acidity of oils.

Traditional technology for alkaline refining of cotton oils is not always effective, etc. for processing of high-dark and impermeable black oils output and the quality of the resulting products are below standard. At the same time, there is a great loss of valuable oil, reagents, energy, etc. , [16].

Agronomic breeding has yielded varieties of cotton that are devoid of glands that produce gossip, but these varieties are usually not grown because they are less productive and more vulnerable to insect attacks.

It has been studied that Gossipol inactivates the human immunodeficiency virus (HIV) in the system in vitro, [17].

In research, it has been shown that biologically active plant compounds have therapeutic properties that are used as a drug, as well as as ingredients for functional food products and nutrients. Although qualitative and quantitative studies of plant extract bioactive compounds are important for establishing their claimed therapeutic properties, less focus is placed on the effects and interactions of process conditions (extraction) to obtain the desired biologically active compounds,[18].

Research [19] presents an update of the use of DES (Data Encryption Standard) in the conversion of biomass as renewable sources. The review is intended to cover as much as possible current research and applications of DES and to offer opinions for the expansion of DES applications rather than focusing on the physio-chemical bases of the new DES. The future of these solvents is bright, but further research and efforts are needed to better understand the future of sustainable resources.

The removal of the gossypol from cottonseeds could significantly improve the use of this valuable protein resource for global food and fibre security as we enter an era of uncertain climatic conditions,[20]. After reviewing the review of studies, we worked to create a new way to control gossypol content in cottonseed.

## Materials and methods

To understand how gossypol (C<sub>30</sub>H<sub>30</sub>O<sub>8</sub>), [1,2,3,4,5,6,7,8, 12, 14, 20], we have conducted a number of experimental studies, analyses, and observations. The reasons for the emergence of the State Sex are described below.

**1. Changes in the soil.** In the soil, where uncultivated plants are found, the amount of hydrogen ions increases and reacts with chlorine to form chloride acid.  $H^+ + Cl^- = HCl$ . The field is gradually coated with chloride acid. The root hairs of such plants dissolve fertilizers that cannot be dissolved or absorbed by the roots of the plants, and dissolve the fertilizer and thus increase the soil hydrogen ion  $H^+$ . If crops are sown without first removing hydrogen ion from the soil, the roots rot, plant growth slows, and they die. Once irrigated, the ripening of the soil is accelerated if the soil is not ploughed in time, and the soil quickly hardens. In a cotton field, where uncultivated plants grow, there are strong changes in the seed and fiber of the cotton: the content of gossypol in the seed increases. The average value of gossypol in a seed, if we conditionally take it equal to 0.76%, [21], then 0.23% of them are formed due to changes in the soil produced by uncultivated plants. A change in the composition of the seed by the action of non-self-cultivated plants is called a combinative change. In such cases, uncultivated plants perform a mutagenic role. Mutagenic substances are directly affected by cottonseeds. In order to get rid of combinative changes in cottonseeds, you have to act on it with a biological solution - prepared by mixing cattle manure in water. As a result, 0.23% of the gossypol is synthesized for vitamins and hormones, as well as for strong antibiotics, the characteristics of which should be studied in further studies. To get rid of the hydrogen ion, which contributes to combinative changes in the soil of the Earth, it is necessary to treat the soil with livestock manure. Research results show that if 1 hectare of land is filled with 2 tons of manure once before sowing, then the impact of uncultivated and cultivated grasses becomes invisible. Gossypol, formed by combinative changes, disappears from the seed, the oil becomes healing.

**2. Exposure to mineral fertilizers.** All changes in the structure of the soil of the Earth, and the plants sown on the soil and the yield obtained from these plants, occurring due to the use of mineral fertilizers, can be called ontogenetic or phenotypic. Under the influence of ontogenetic transformations, 0.22% of gossypol is formed in the cottonseed. To get rid of this gossypol, it is enough to soak the seeds in a solution of lamb manure in water.

**3. Impact of the memory of water.** There are a number of reasons leading to the pollution of the water memory. As an example, we can cite one of a number of studies by scientists of the International Islamic University of Malaysia, on the purification of waste dyes from the textile industry, [22]. The pollution of the memory of water has a profound effect on the plant world and on all living things. Strong mutational changes occur in humans and plants. Human health deteriorates, genetic diseases appear, before which medicine is powerless. For example, bone cancer, liver cancer and others. A strong poison appears and increases in the seed of plants. The content of gossypol in cottonseed increases by 1.5%.

This gossypol can be destroyed by soaking the seed in a biological solution, which contains chicken manure and a very small amount of mineral fertilizer, which is used as a catalyst.

The second way is irrigation of plants with water with purified memory. Gossypol is not eliminated

- it is synthesized into hormones and vitamins.

**4. Impact of changing the position of the axis of the earth.** The change in the position of the axis of the earth has a strong effect on the planet Earth, including the change in the content of gossypol in the cottonseed. This change is expressed quantitatively as follows, see Table 1.

Table 1. Influence of changes in factors on the content of gossypol

Nº	Indicators	Quantity, %
1.	Changes in the soil	0.23
2.	Changes under the influence of mineral fertilizers	0.22
3.	Changes under the influence of water memory	0.15
4.	The impact of a change in the position of the axis of the earth	0.11
5.	Atmospheric pollution	0.05
Total		0.76

## Results and discussion

Experimental studies have shown that gossypol, which has appeared under the influence of atmospheric pollution, can be eliminated by soaking in a solution containing three types of natural mineral fertilizers (cattle manure, lamb, chicken droppings, urea and ammonium nitrate at the rate of 60 g each for 50 liters of water ). Gossypol, which appeared under the influence of changes in the position of the axis of the earth, as studies have shown, cannot be liquidated. At the same time, research continues on the elimination of such gossypol. The advantage of these works is that gossypol is not separated from the composition of the semen, it turns into vitamins B<sub>1</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>7</sub>, B<sub>9</sub>, B<sub>11</sub> and two hormones. This oil can be used medicinally. We have developed methods for the treatment of sowing cottonseeds with dewlap, (with residual fiber) and without fiber. Experiments show that when processing with special biological solutions, profound changes occur in the composition of the cottonseed. Especially in the content of gossypol. This ensures genetic changes in the cottonseed. The properties of seeds change over time.

The biological solution in seven variants was prepared as follows: 1-option - cattle and lamb manure 15 kg each, poultry droppings 9 kg, urea and ammonium nitrate at the rate of 30 g each per 50 l of water, option 2 - cattle and lamb manure 17 kg each, poultry droppings 9 kg, urea and ammonium nitrate at the rate of 60 g each for 50 liters of water, option 3 - cattle and lamb manure at 21 kg, poultry manure 13 kg, urea and ammonium nitrate at the rate of 90 g each for 50 liters of water. In the third, fourth, fifth, sixth and seventh variants, the content of the components was increased while maintaining the multiplicity of each of them.

Next, 14 liters of water were poured into the barrel and the components of the 1st variant were placed on top. After 10 minutes after mixing and settling, when all the components sank to the bottom, the solution was poured into the first canister, and the remainder was transferred to another barrel for use as fertilizer. 14 liters of water were poured into the barrel, freed from the components, and the components of the 2nd variant were placed on top. After 15 minutes after mixing and settling, when all the components sank to the bottom, the solution was poured into a second canister, and the remainder was transferred to a barrel for use as fertilizer. 14 liters of water were poured into the barrel, freed from the components, and the components of the 3rd variant were placed on top. After 20 minutes after mixing and settling, when all the components sank to the bottom, the solution was poured into a third canister and the remainder was transferred to a fertilizer barrel. Thus, we have prepared a solution in seven variants, in seven cans. The effect of these solutions on the content of gossypol was studied as follows: per 3 kg of cottonseed in three variants - with residual fiber, without fiber and treated with chemicals, prepared for sowing the seed using a watering can was sprayed with 300 g of the solution contained in the first canister. After soaking, a black textile material was applied and covered with cellophane wrap on top. After waiting in this position for 5 minutes, they removed the black material and cellophane and dried the seed.

Moreover, under the rays of the sun, the drying time was 15 minutes, in a shady place - 20 minutes. After drying, the content of gossypol in each of the samples was analyzed. Therefore, we checked the effect of each of the solutions with different concentrations, which is in 7 canisters. Table 2 shows the results of laboratory analysis of the content of gossypol in cotton seeds of varieties C-6524 and Akkurgan-2 grown in Kasansay, Turakurgan and Namangan districts of the Namangan region.

Table 2. Indicators of gossypol content in cottonseeds

№	Districts of the region	The amount of free gossypol, %	Normative document
1	Kasansay district	0.174	ГОСТ 13979.11
2	Turakurgan region	0.204	ГОСТ 13979.11
3	Namangan region	0.21	ГОСТ 13979.11
4	Experimental field No. 1	0.151	ГОСТ 13979.11
5	№2	0.32	ГОСТ 13979.11

The cottonseeds shown in Table 2 were divided into two groups: seed with fiber, seed without fiber. These seeds were processed by soaking in a solution containing three types of natural mineral fertilizers (cattle and lamb manure, chicken droppings) [23, 24, 25]. Seeds that were treated with herbicides were also analyzed. Table 3 shows the summary results of laboratory analyzes of the content of gossypol after treatment with a biological solution.

Table 3. Indicators of the content of gossypol in cottonseeds after treatment with a biological solution

№	Factors	Gossypol content						
		Experiments						
1	Seeds without fiber	0.63	0.123	0.075	0.086	0.216	0.192	0.092
2	Seeds with fiber	0.117	0.110	0.063	0.152	0.105	0.123	0.07
3	Herbicide-treated seeds (control)	0.103	0.107	0.148	0.125	0.071	0.151	0.151

The essence of the method for determining the quantitative content of gossypol consists in the extraction of free gossypol from the analyzed product with a mass fraction of moisture not exceeding 7% with anhydrous acetone and the subsequent quantitative determination of gossypol by the photometric method in accordance with existing standards in accordance with GOST 13979.11.

The diagrams clearly show the value of free gossypol in the bio-solution-treated seed with fiber. Fig. 1, the value of free gossypol in bio-solution-treated seed without fiber, Fig. 2, the value of free gossypol in herbicide treated seeds, Fig. 3, (control).

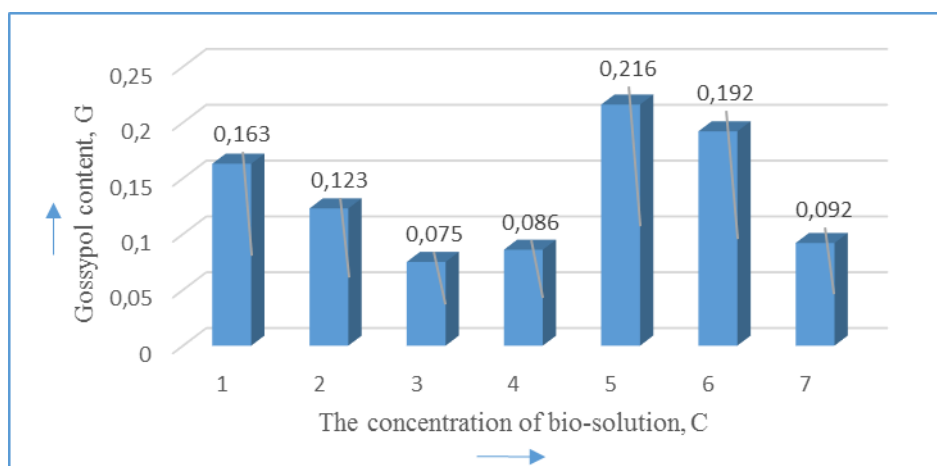


Fig. 1. The value of free gossypol in bio-solution-treated seed without fiber

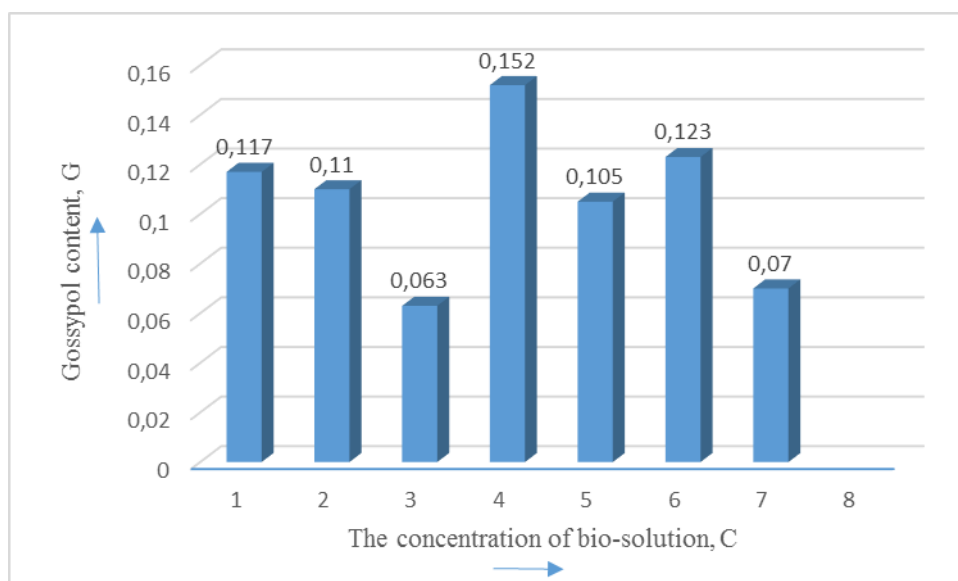


Fig. 2. The value of free gossypol in bio-solution-treated seed with fiber

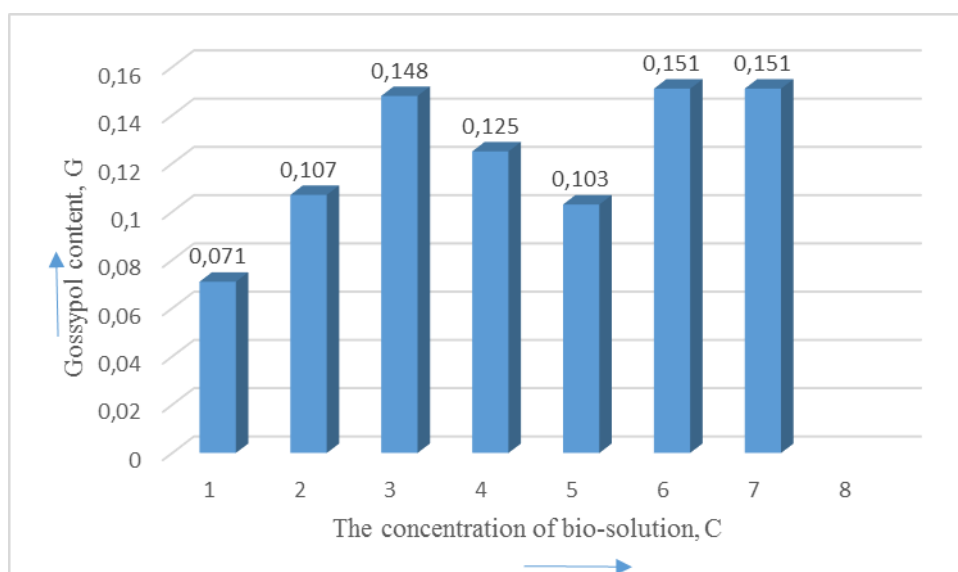


Fig. 3. The value of free gossypol in herbicide-treated seeds (control).

The diagram shows that in cottonseeds processed with herbicides (Fig. 3), the amount of gossypol

decreases rapidly, in comparison with others, it is consumed for decomposition of the poison in the seed.

At low air temperatures and when the moisture content in the seed is exceeded, gossypol spreads over the volume of the seed and the seed decomposes, accompanied by a peculiar heavy odor. Gossypol does not turn into a useful substance, on the contrary, the content of the poison increases. The diagram of a seed untreated with herbicides shows (Fig. 2, seeds with fiber) that, with a small amount of biological solution, as well as with a maximum content, the presence of gossypol will be minimal, (0.063% and 0.07%), because gossypol begins to move towards the surface of the seed, and before reaching the surface, under the influence of a special biological solution, it turns into vitamins, i.e. the main part of gossypol in the gossypol nodule turns into vitamins B9, B12. With an increase in moisture, two opposing processes continue, and the content of gossypol will be large. With an increase for solution, the content of gossypol will be relatively large. The high content of the biological solution increases evaporation, the temperature begins to decrease, and the breakdown of gossypol into vitamins decreases. With the passage of time, the temperature of the seed rises, the decomposition of gossypol increases the temperature of the biological solution, the breakdown of gossypol into vitamins increases, and the formation of vitamins increases.

Gossypol was analyzed every two months, the analysis was carried out 3 times, and as a result, a result was obtained where the content of gossypol was reduced to zero. The diagram of a seed untreated with herbicides shows (Fig. 1, seeds without fiber) that the content of gossypol first dropped to the level of 0.075%, and then gradually increased over time and at the end of the experiment was 0.092%. Comparative analysis shows that in seed with drooping (with fiber), the process of decomposition of gossypol occurs better, this indicates the need for the presence of fiber in the seed. Apparently, the fibers improve the penetration of the solution into the inner cottonseed. The seed oil obtained from the cotton crop, sown after pretreatment with a biotechnological method, has antidote properties, i.e. it can be used in the food industry, in medicine.

### Conclusions:

Gossypol is a poison to a living organism as a toxic substance on the one hand, on the other hand - a raw material for the creation of high-efficiency drugs with a wide range of effects;

The gossypol content increases due to environmental degradation;

A novel biotechnological method for controlling the content of the gossypol leading to its decay into vitamins is proposed: this means that the cotton oil obtained from the cotton seed, which is treated in a biotechnological manner, can be used as a healing agent;

The prospects are open for the use of cotton products grown by a new biotechnological method in medicine, the food industry and the military sphere.

**Confirmation.** All analyzes were carried out in the laboratory of the Namangan Regional Center for Sanitary and Epidemiological Control, all the indicators entered in the tables and figures are documented, (NSS.Uz.NSO.197.n.2266725).

The author has documents certified by the seal of the controlling organization.

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