of polyphenols had risen but the level of gossypol pigments with free aldehyde groups had not changed.

3. The changes in the amounts of individual unsaturated fatty acids had a complex nature.

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INFLUENCE OF WILT INFECTION ON THE GOSSYPOL PIGMENTS OF SEEDS AND ROOTS OF A COTTON PLANT OF THE VARIETY TASHKENT-1

I. P. Nazarova, A. I. Glushenkova, N. T. Ul'chenko, and G. P. Moiseeva

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A study has been made of the gossypol pigments of the seeds and roots of a cotton plant of the variety Tashkent-1 infected with wilt in comparison with a healthy plant. The amount of gossypol in the infected plant was lower than in the healthy plant. In the diseased plant, gossypurpurin was concentrated in the roots, and in the healthy plant it was concentrated in the seeds. Gossypol possessing optical activity was detected in the seeds and roots of both the healthy and the diseased plants.

The resistance of the cotton plant to the causative agent of verticilliaceous wilt depends to a considerable degree on phenolic compounds and, above all, on gossypol, which participates in the protective reactions of the plant against attack by wilt [1].

We have made a comparative study of the gossypol pigments of the seeds and roots of a healthy cotton plant of the variety Tashkent-1 and of one attacked by verticilliaceous wilt.

Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent.

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TABLE 1. Extraction of Gossypol from the Seeds and Roots of a Cotton Plant of Variety Tashkent-1

Sample	Extracts										
	hexane			chloroform					chloroform–methanol		
	yield %	gossypol		yield %	gossypol		gossypol-like		yield %	gossypol	
		%	3		%	g	%	g		%	g
Seeds											
I	16,38	0,28	0,05	3,29	17,04	0,56	0,83	0,03	1,58	1,07	0,02
II	12,28	0,26	0,03	2,58	19,80	0,51	Tr.		1,23	1,30	0,02
Roots											
I	0,32	24,44	0,08	1,05	21,82	0,23	1,08	0,01	1,57	1,29	0,02
II	0,26	6,13	0,02	0,65	30,85	0,20	6,92	0,04	1,82	1,86	0,03

The amount of free gossypol in the seeds of the healthy plant (I) was 0.64%, and in the diseased plant (II) 0.57%, and the corresponding figures for the roots were 0.33 and 0.26%, respectively, i.e., the amount of gossypol in the healthy plant was higher than in the diseased plant, which can be explained by the oxidation of the polyphenol in response to the disease.

The complex of gossypol pigments was extracted stagewise by successive extraction: first with hexane to separate the neutral lipids, and then with chloroform into which the bulk of the gossypol passed, and finally with chloroform–methanol (2:1). This enabled the changes in gossypol that take place in extractants containing methanol to be avoided and thereby enabled the pigments to be isolated in the native form [2].

Table 1 gives the results of such an extraction of gossypol from 100 g of the seeds and roots of the cotton plant. The total yield of extracts both from the seeds (21.25%) and from the roots (2.94%) of the healthy plant was somewhat higher than from the diseased plant (16.09 and 2.73%, respectively).

The hexane extracts of the roots differed from those of the seeds both by their lipid compositions and by their gossypol contents. The lipids of the seeds consisted mainly of triacylglycerols, while the lipids of the roots consisted of wax esters, triacylglycerols, free fatty acids, sterols, and triterpenols. The amounts of gossypol in the hexane extracts in the seeds of both plants were almost the same, at only 0.26-0.28%, while the extracts of the roots contained a considerable amount of gossypol, that in (I) being four times greater than in (II).

Table 1 shows that the bulk of the total amount of gossypol in the samples passed into chloroform: for seeds (I) – 87.5% (0.56 g of gossypol from a total amount of 0.64 g in 100 g of seeds (I)); (II) – 89.4%; and for the roots 69.7 and 76.9%, respectively. Further extraction with chloroform–methanol removed the remaining small fraction of the gossypol, and in this process, because of the presence of methanol, the formation of artefactual gossypol-like products was possible [2]. Therefore to study the natural gossypol pigments we used the hexane extracts of the roots and the chloroform extracts of the seeds and roots of both plants.

In the investigation of the hexane extracts of the roots, attention was attracted by the high gossypol content, particularly for the healthy plant (24.44%). This is unusual, since it is known that gossypol is sparingly soluble in hexane (0.12%) [3]. We assumed that one of the reasons for such an anomaly may be the presence, together with the racemic form, of optically active (+)-gossypol, which possesses a higher solubility in hexane [4]. And, in actual fact, a determination of the specific rotation confirmed this: for the gossypol present in hexane extracts of the roots of the healthy plant $[\alpha]_D + 12°$, and those of the diseased plant $[\alpha]_D + 10°$.

The gossypol from the chloroform extract of the seeds and roots of (I) and (II) was reprecipitated with hexane. It was observed with the aid of ATLC that gossypol was present both in the precipitate and in the filtrate.

Optically active gossypol was detected in the filtrates of the chloroform extracts both of the seeds and of the roots of both plants, with $[\alpha]_D + 21$ and 28°, respectively, for seeds (I) and (II) and $[\alpha]_D + 15$ and +33°, respectively, for roots (I) and (II).

In addition to gossypol, gossypurpurin was found in the chloroform extract, and this was precipitated from hexane together with the gossypol. It can be seen from Table 1 that the highest level of gossypurpurin – 6.92% – was found in the extracts of roots (II), this amounting to 0.04% of the weight of the roots, while in roots (I) the level was four times lower, 0.01% (with 1.08% in the chloroform extract). In the seeds the opposite pattern was observed: in (I) 0.03% of gossypurpurin (0.83% in the extract), and in (II) only trace amounts; i.e., in the diseased plant gossypurpurin was concentrated in the roots and in the healthy ones it was concentrated in the seeds.

In the complex of gossypol pigments from the chloroform extract that had been freed from accompanying lipids it was established with the aid of TLC and UV spectroscopy that the qualitative compositions of the pigments both of the seeds and of the roots (I) and (II) were identical and that they consisted mainly of gossypol (R_f 0.55; $\lambda^{C_2H_5OH}$ 376 nm) and of gossypurpurin (R_f 0.24; λ^{CHCl_3} 530, 565 nm).

Thus, in the seeds and roots of a cotton plant infected with wilt a fall in the amount of gossypol as compared with a healthy plant was observed but the quantitative compositions of the gossypol pigments of (I) and (II) were similar.

EXPERIMENTAL

Specimens of healthy and wilt-affected cotton plants of the variety Tashkent-1 were provided from the experimental section of the G. Z. Zaitsev Institute of Selection and Seed Production by R. G. Kim.

The seeds were comminuted in an electric coffee mill, and the roots were cut into pieces with a size of 0.3-0.5 cm. The material was extracted by five steepings with the appropriate solvents at room temperature.

The neutral lipids of the extracts under investigation were identified on the basis of their migrational characteristics in a thin layer of silica gel in comparison with model classes of lipids.

The complex of gossypol pigments from the chloroform extracts was freed from phospholipids by reprecipitation with cooled acetone, and from residual amounts of neutral lipids and glycolipids by CC on silica gel.

The neutral lipids were eluted with a mixture of the solvents hexane and ether in which the amount of the latter was brought to 40%, and the gossypol by the same solvent system in a ratio of 1:1, while the glycolipids remained in the column.

TLC on Silufol and silica gel was carried out for the neutral lipids in hexane–ether systems with ratios of 9.5:0.5, 7:3, and 1:1, and for gossypol and gossypurpurin in the benzene–methanol (20:5) system.

The amount of gossypol was determined by the para-anisidine method [5] and that of gossypurpurin as described in [6].

Because of their dark yellow coloration the gossypol solutions under investigation absorbed strongly, which made it difficult to determine the angle of rotation on a polarimeter. The specific rotation was therefore determined on a JASCO J-20 spectropolarimeter. The concentration of the solutions was 5 mg/ml and the thickness of the cell 1 cm. Ethanol was used as solvent.

SUMMARY

1. In the seeds and roots of a cotton plant infested with verticilliaceous wilt the amount of free gossypol was lower than in the same parts of a healthy plant.

2. Gossypurpurin in an amount of 0.03-0.04% was concentrated in the seeds in the case of the healthy plant, and in the roots in the case of the diseased plant.

3. Gossypol possessing optical activity was found in extracts of the seeds and roots of both the healthy and the diseased plants.

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LIPIDS OF THE PRODUCTS OF PROCESSING OF COTTON SEEDS

M. Talipova, T. V. Chernenko, and A. I. Glushenkova

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The free, bound, and strongly bound lipids of the crushed seeds, pulp, husks, and meal have been characterized. It has been shown that the bound and strongly-bound lipids differ from the free lipids by a higher level of saturated acids. The acid numbers of the bound lipids are 5-6 times higher than those of the free lipids.

In the production of vegetable oils by the current technology from oil seeds, their lipid composition changes [1]. We have investigated the free (F), bound (B), and strongly-bound (SB) lipids in the processing of cotton seeds: the crushed seeds (I), the pulp (II), the husks (III), and the meal (IV), samples of which were obtained in the Tashkent Oils and Fats Combine. Below we give some indices of the products of the processing of cotton seeds:

Index	Amount, %			
	I	II	III	IV
Free lipids	28.02	28.40	18.00	0.91
Bound lipids	3.24	3.40	3.60	3.71
Strongly bound lipids	0.28	0.32	0.33	0.36
Moisture content	8.12	4.70	3.90	10.29
Free gossypol	1.24	0.31	0.24	0.08

The free lipids made up 88.9% of the mass of the lipids in the crushed seeds, the proportions of bound and strongly-bound lipids being 10.2 and 0.9%, respectively.

In the course of the industrial process for the extraction of the oil, the amount of free lipids in the seed-processing products naturally falls sharply, while the amount of bound and strongly-bound lipids changes only slightly. Some increase in the amount of free lipids is explained by the fact that in the moist heat treatment of (I) the lipids interact with the protein molecules [2].

The decrease in the amount of free gossypol is also explained by its capacity for taking part in a chemical interaction with many components of cotton seeds.

The free and bound lipids isolated from (I)-(IV) were analyzed as described in [3]. The neutral lipids from all the samples contained the following set of classes: traces of hydrocarbons, sterol esters, triacylglycerols, epoxyacylglycerols, free fatty acids (FFAs), free sterols, diacylglycerols, and traces of monoacylglycerols. The polar lipids isolated from the free lipids of all the samples investigated contained glyco- and phospholipids. These were monogalactosyldiglycerides, digalactosyldiglycerides, sterol glycosides, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, and

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