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STRUCTURAL FEATURES OF CHITIN FROM ARAL CRUSTACEANS AND USE OF CHITOSANS BASED ON IT

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Structural features of chitin from Aral crustaceans and chitosan based on it are examined using x-ray diffraction and IR spectroscopy. Their biological activities on encapsulated cotton seeds are determined.

Key words: chitin, chitosan, structure, biological activity.

Chitin and its derivatives that are obtained from various biological specimens are a continuing topic of current research. The mechanisms of their biological synthesis, modification, structure, properties and activity have been studied [1-12]. We previously isolated for the first time chitin from the shells of the Aral crustacean *Palaemon elegans* and synthesized chitosan and the sodium salt of carboxymethylchitosan from it. The potential of using the synthesized products as biologically active stimulators and fungicides for treating cotton seeds was demonstrated [13].

We investigated the structural features of chitins and chitosans obtained from Aral crustaceans and also discovered their physiological activity. Therefore, we studied samples of chitin and chitosan that were synthesized from local specimens, the shells of the Aral crustaceans *Palaemon elegans* (shrimp) and *Phitrophanapeus harrissi* (crab), using x-ray diffraction, microscopy, and IR spectroscopy. We also studied the production of polymeric coatings for cotton seeds using encapsulation. It is noteworthy that the structures of crab and shrimp chitins are identical.

The microscopy studies showed that chitin and chitosan, regardless of their origin, are white (crab) or brown (shrimp) powders with various particle sizes and shapes (fibers, films, and sometimes plates). The crude crab and shrimp chitin is rather fine (50-500 μm particle size). The particle size increases after purification and conversion to chitosan. Film-like particles were observed to be smooth and sometimes structured. Randomly oriented threadlike features are seen over the whole surface. These are seen more clearly in chitosan and especially in crab chitin. Chitin and chitosan samples shine poorly under crossed Nicol prisms, suggesting that the samples are anisotropic owing to their orientation or crystallinity (Fig. 1).

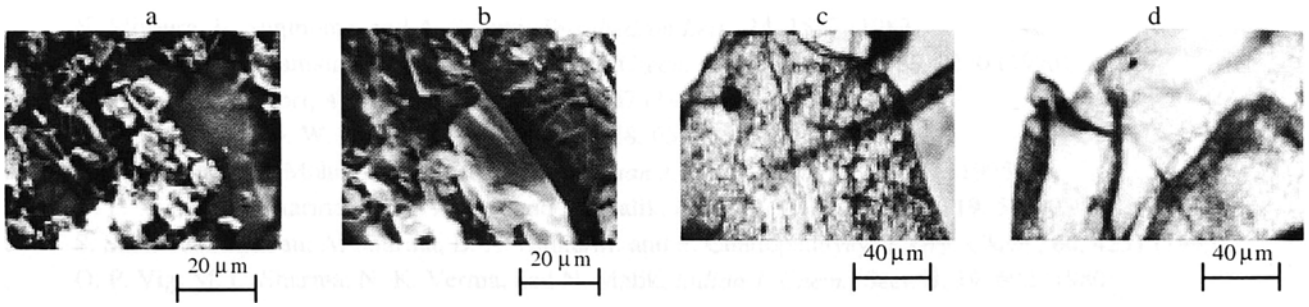

Fig. 1. REM (*a*, *b*) and optical (*c*, *d*) images of samples of crude crab chitin (*a*), purified crab chitin (*b*), purified shrimp chitin (*c*), and shrimp chitosan (*d*).

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TABLE 1. Effect of Polymeric Chitosans on Seed Germination

Variant	Growth energy, %	Laboratory fertility, %	Rate of germination, % on			Field fertility, %
			05.02.97	05.04.97	05.06.97	
Control	92	93	28.5	62.0	76.5	63.0
Industrial chitosan	97	98	43.0	81.0	95.0	72.4
Synthetic chitosan	89	91	61.5	85.0	93.5	76.3
M=8000	94	95	66.0	80.5	96.5	76.6
M=300000	95	96	55.0	79.0	90.0	70.3

TABLE 2. Effect of Chitosans on Gummosis Infection and Root Rot

Variant	Fertility, %	Growth energy, %	Root rot, %	Gummosis, %
Control	61	27	16	44
Industrial chitosan	69	41	12	29
Synthetic chitosan	77	43	10	18

TABLE 3. Effect of Chitin Derivatives on Seed Quality and Cotton Harvest (1996 Experiment)

Variant	Fertility, %	Rate of germination		Field fertility, %	Cotton harvest, 1-2 collection, centner/hectare
		10.5	12.5		
Control	93	6.5	31.0	36.0	32.2
Industrial chitosan	93	7.0	33.5	43.9	30.0
Synthetic chitosan	95	18.5	55.5	50.1	34.3

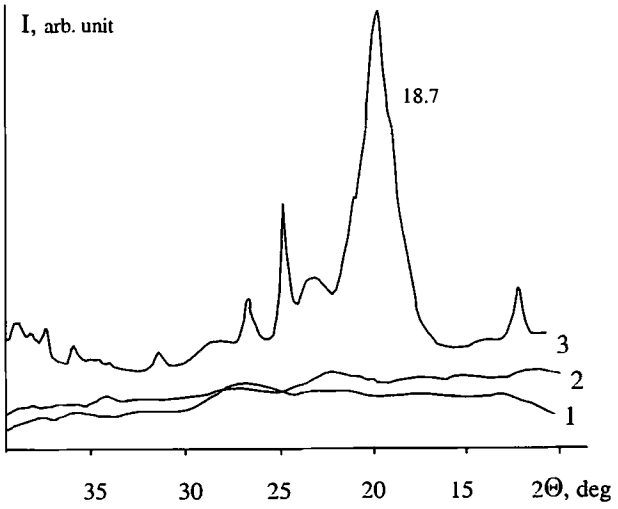

Fig. 2

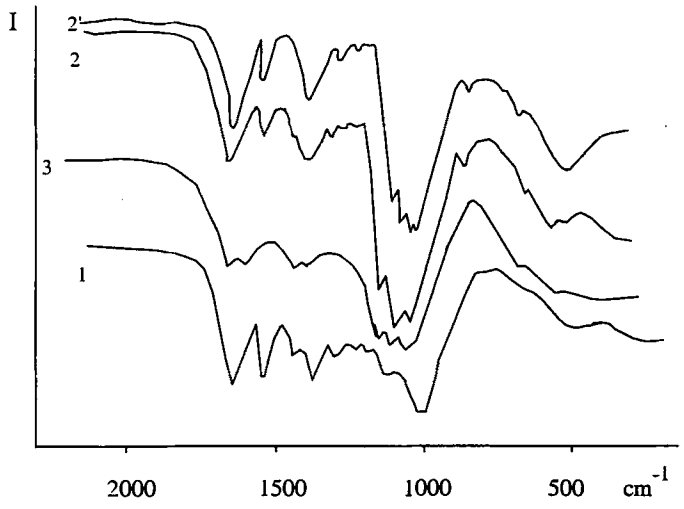

Fig. 3

Fig. 2. X-ray diffraction patterns of crude crab (1), crude shrimp (2), and purified shrimp chitin (3).
Fig. 3. IR spectra of crude crab (1), purified shrimp (2), purified crab chitin (2') and shrimp chitosan (3).

X-ray diffraction studies showed that the starting material for extracting chitin is an amorphous material (Fig. 2). The diffraction pattern is an almost straight line with a small maximum that is probably due to an accidental impurity. The sharp maximum at $2\Theta = 29.2^\circ$ in crab chitin is probably due to the inclusion of inorganic impurities. The crystal structure of chitin is clearly visible after accompanying substances are removed. The diffraction pattern has four maxima at $2\Theta = 18.7$, 22.5, 26.3, and 38.5° and a small hump at 12°. The peak at 18.7° is very strong and sharp. The crystal structure changes greatly after removal of the acetyl groups from chitin (conversion to chitosan). The strongest peak in the range $18\text{-}19^\circ$ remains but shifts slightly to $2\Theta = 19^\circ$. The intensity markedly decreases. The remaining maxima are not observed. Only a weak and broad maximum is observed at $2\Theta = 39^\circ$.

Thus, removing the acetyl groups causes an extensive structural rearrangement of chitosan that decreases the degree of crystallinity, i.e., makes it amorphous. IR spectroscopic studies (Fig. 3) reveal in the spectrum of chitin absorption bands of an amide at 1665 cm^{-1} (C=O stretching). The absorption bands of the amide almost disappear in the IR spectra of chitosan, consistent with the transformation of chitin into chitosan. The peak from $-CH_2-$ deformation at 1460 cm^{-1} also broadens and decreases slightly in frequency, in agreement with the literature [14].

Biological tests of chitin and its derivatives showed that the germination rate of seeds treated with synthesized chitosan (1.0%) exceeded significantly that of controls (by almost 2-3 times). The field germination also increased by 10-20% (Table 1). Shrimp chitosan (*Palaemon elegans*) possesses distinctly stimulating properties. The growth energy and fertility increased by 16%. Infection by root rot and gummosis decreased 1.5-2.5 times relative to the control (Table 2). The rate of appearance of leaves increased by 2-3 days. This also indicates that the preparation is biologically active. The germination rate of seeds treated with synthetic chitosan (1%) increased significantly compared with a control (by almost 3 times). The field germination increases by 8-14% (Table 3). Preliminary results for two species suggest that the average harvest is 2.1-3 centner/hectare greater than that of the control.

Thus, the results reveal certain structural features of chitin from Aral crustaceans and its derivatives and their biological activity.

EXPERIMENTAL

Microscopy studies of the external shape and size of specimens were performed using an MBI-6 optical microscope and a REM-200 scanning electron microscope in transmitted and polarized light at 380-500X magnification. Diffraction patterns of the crude material, chitin extracted from it, and chitosan synthesized from it were obtained on a DRON-3M diffractometer in the range $2\Theta = 9\text{-}42^\circ$. IR spectroscopic studies of chitin and chitosan were carried out on a Specord IR-75 spectrometer in pressed KBr pellets. The effect of polymeric systems containing synthesized chitosan and industrial chitosan of various molecular weights on the growth and development of cotton was measured at the Uzbek Research Institute of Selection (C-6524 variety from Namangan factory). Seeds treated with formalin were the control. Cotton was harvested twice. Experiments for estimating the ability of synthesized chitosan from *Palaemon elegans* and industrial chitosan in identical concentrations to counteract gummosis and root rot were carried out on growing specimens at another Uzbek institute. The experiment was repeated four times (Fergana-3 seeds). Uninfected seeds served as the control.

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