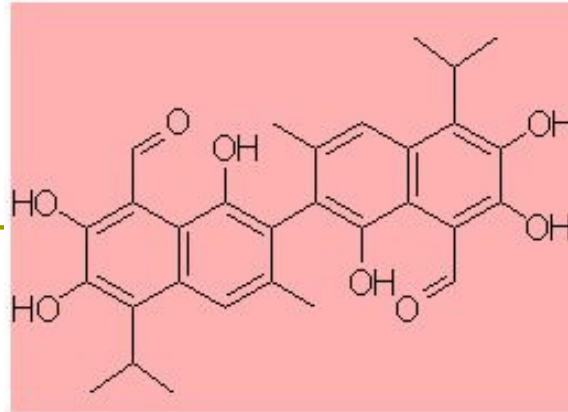


Utilization of the Cotton Terpenoid Gossypol Against *Staphylococcus aureus*



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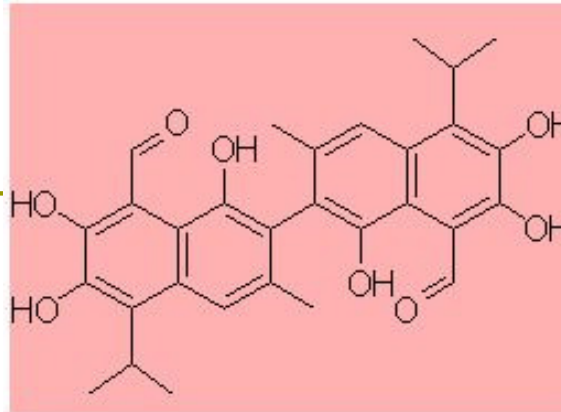
Gossypol



- Cotton's First Line of Defense
- Protects Against Pathogens and Predation



Gossypol



Gossypol: Two Enantiomers

- (-)-gossypol: Cytotoxic & suppresses sperm production.
- (+)-gossypol: Less Toxic & No sperm suppression

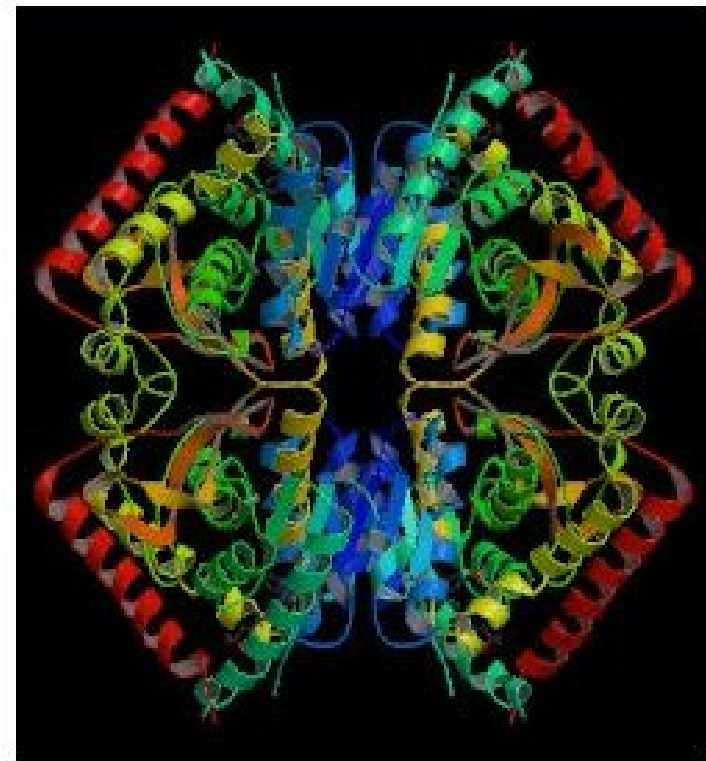


Gossypol's History in Human Medicine



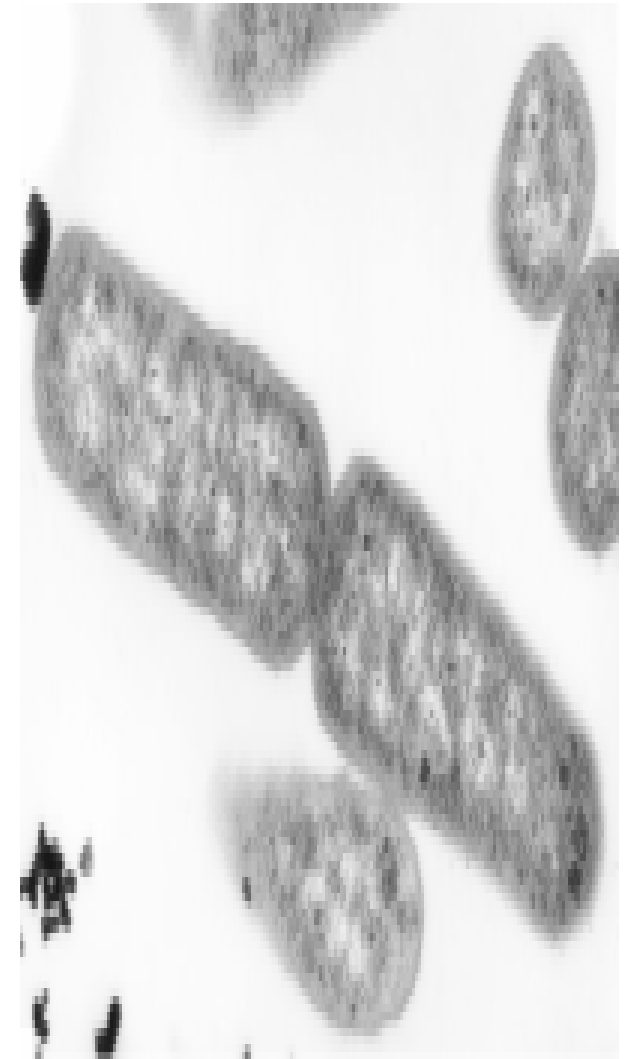
- ❑ Male Contraceptive
- ❑ Kills Tumors
- ❑ Anti-malarial
- ❑ Retroviral Inhibitor:
HIV and Influenza

**Protein Data Bank (PDB)
Gossypol Derivative
Structures Complexed to
Plasmodium falciparum
Lactose Dehydrogenase**



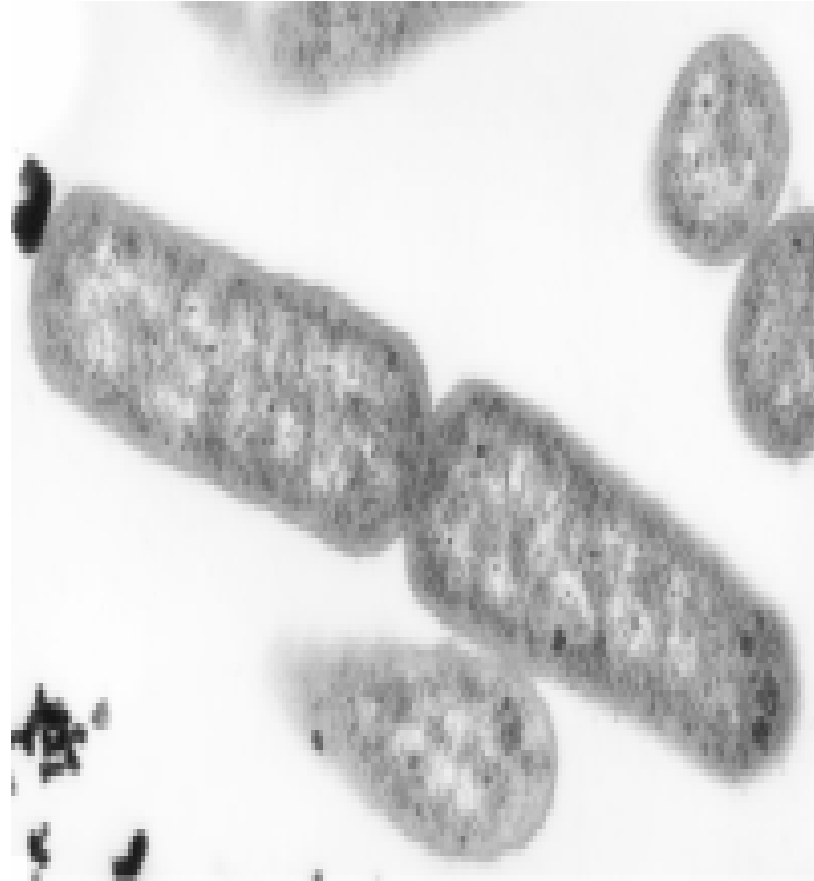
Eating Cotton Seed Protects Against Infection

- ❑ Prevented Septicaemia in Catfish
- ❑ Effective against Gram-negative *Edwardsiella ictaluri*.
- ❑ Reduced Bacterial Colonies.
- ❑ Complete killing not reached until 1000mg/L gossypol



Gossypol against *Edwardsiella ictaluri*

- ❑ (+)-Gossypol More Effective
- ❑ (+)-Gossypol Safer
- ❑ Cottonseed: Protein Rich
- ❑ Prevents Bacterial Infections
- ❑ Reduce Agricultural Antibiotic Use



Gossypol's Effectiveness in Killing Bacteria

- Gossypol Against Human Pathogens
- Effective Against Gram-positive
- MICs ~3-30 mg/L Against Gram-positive
- MIC 250 mg/L Against Gram-negative *E. coli*
- MDR Inhibitors: Increased Gram-negative Effectiveness

MIC ($\mu\text{g/ml}$)

Antimicrobial and addition ^a	MIC ($\mu\text{g/ml}$)													
	<i>S. aureus</i>	<i>S. aureus</i> <i>norA</i>	<i>B. mega-</i> <i>terium</i> ^a	<i>E. coli</i>	<i>E. coli</i> <i>tolC</i>	<i>P. aeri-</i> <i>ginosa</i> PAO1	<i>P. aeri-</i> <i>ginosa</i> <i>melAB</i>	<i>S. enterica</i> serovar Typhi- murium	<i>P. syrin-</i> <i>gae</i>	<i>X. cam-</i> <i>pestris</i> ^b	<i>A. tume-</i> <i>facians</i> ^c	<i>E. rha-</i> <i>pontici</i>	<i>E. caro-</i> <i>tovera</i> ^c	<i>S. meli-</i> <i>loti</i> ^b
Gossypol	3.12	1.95	3.91	250	125	1000	500	500	31.25	125	250	500	250	3.91
MC ₂₀₇₁₁₀	3.12	1.95	3.91	62.5	7.81	250	250	125	31.25	7.81	125	15.65	125	0.98
INF ₂₇₁	32	7.81	15.65	250	62.5	500	500	500	31.25	62.5	125	125	125	0.98
MC ₂₀₇₁₁₀ + INF ₂₇₁	6.25	6.25	7.81	31.25	31.25	250	250	62.5	15.75	3.12	62.5	62.5	62.5	0.98

^a MC₂₀₇₁₁₀ was added at a final concentration of 20 $\mu\text{g/ml}$, and INF₂₇₁ was added at a final concentration of 10 $\mu\text{g/ml}$ except where stated otherwise. All MIC determinations were performed in triplicate.

^b The final concentration of MC₂₀₇₁₁₀ at 10 $\mu\text{g/ml}$ and the final concentration INF₂₇₁ at 5 $\mu\text{g/ml}$ were at least two- to four-fold lower than those inhibiting growth by these compounds alone.

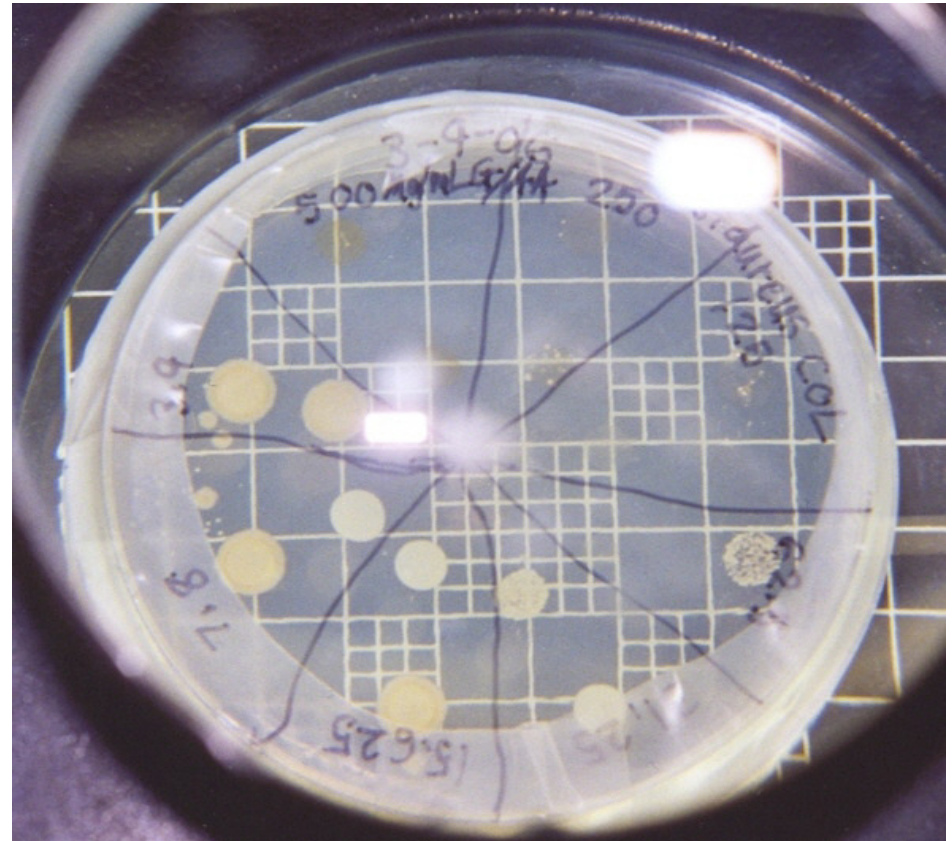
^c MC₂₀₇₁₁₀ at 5 $\mu\text{g/ml}$ and INF₂₇₁ at 2.5 $\mu\text{g/ml}$.

^d ND, not determined.

Taken from Tegos *et al.* (2002) Table 3

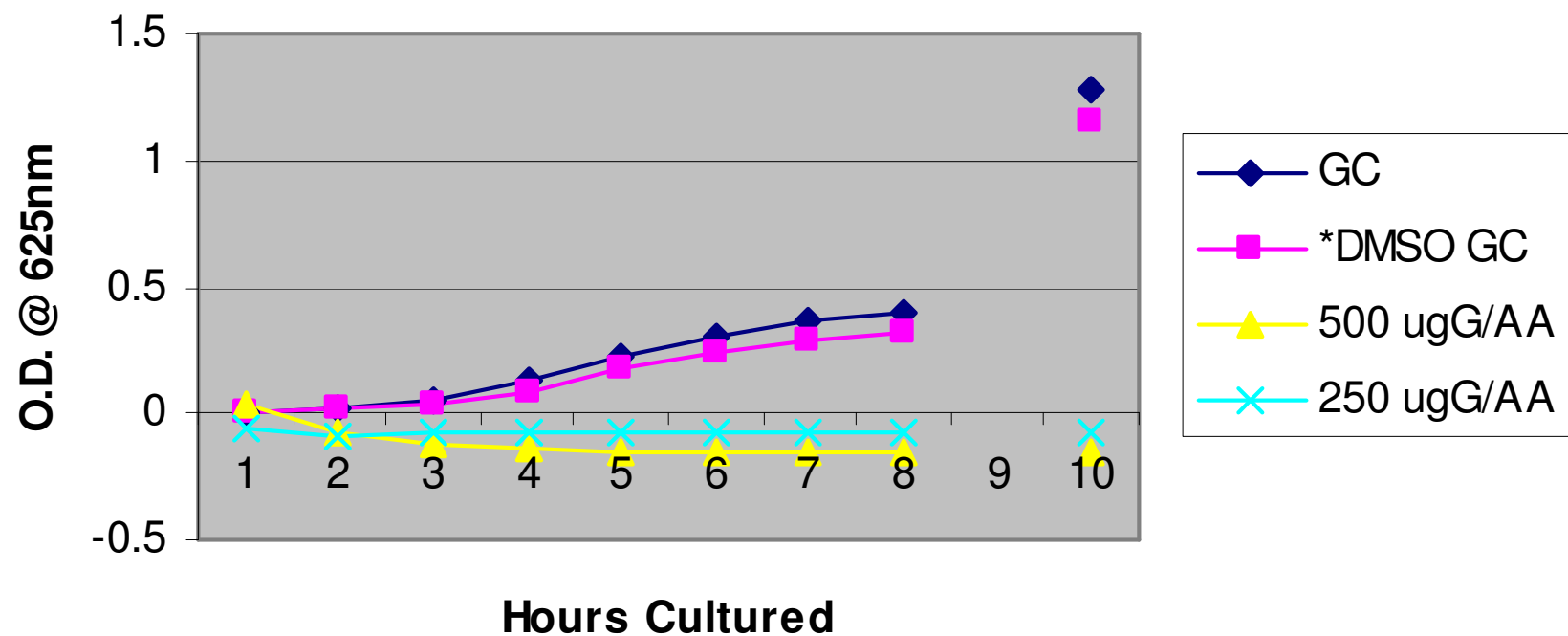
Rationale for Testing Gossypol Against *Staphylococcus aureus*

- MIC Against *S. aureus* of 3.12 mg/L
- Potential as Antibiotic
- My Testing Against *S. aureus* SH1000/COL
- Preliminary Results: Minimal Bactericidal Concentration (MBC) ~250 mg/L
- SH1000 grows faster than COL
- COL likely more resistant to Gossypol



S. Aureus SH1000 Growth Curve

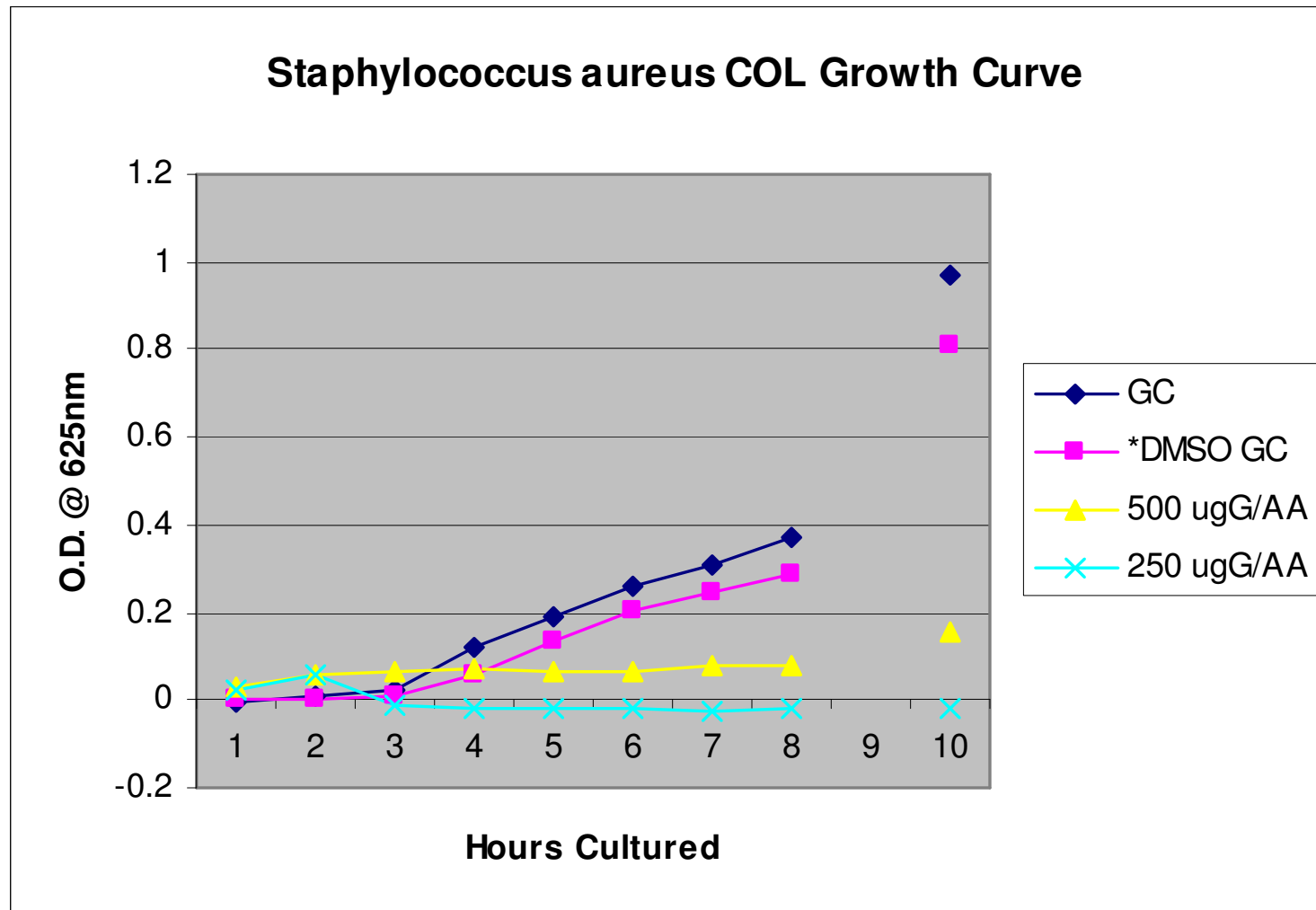
Staphylococcus aureus SH1000 Gossypol/Acetic Acid Growth Curve



S. Aureus SH1000 Minimal Bactericidal Concentration (MBC) Results

SH1000				(*~500ugG/AA)			
Hour GC=Growth Control				*DMSO GC			
	10 ⁰	10 ⁻²	10 ⁻⁴	10 ⁰	10 ⁻²	10 ⁻⁴	
4	TNTC	TNTC	65	TNTC	TNTC	64	
5	TNTC	TNTC	81	TNTC	TNTC	87	
6	TNTC	TNTC	90	TNTC	TNTC	105	
7	TNTC	TNTC	93	TNTC	TNTC	100	
8	TNTC	TNTC	100	TNTC	TNTC	103	
24	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	*TNTC=Too Numerous To Count
SH1000				SH1000			
Hour 500ug G/AA				250ug G/AA			
	10 ⁰	10 ⁻²	10 ⁻⁴	10 ⁰	10 ⁻²	10 ⁻⁴	
4	0	0	0	0	0	1	
5	0	0	0	0	0	0	
6	0	0	0	0	0	0	
7	1	1	0	0	0	0	
8	17	1	0	0	0	0	
24	0	TNTC	TNTC	0	TNTC	TNTC	

Staphylococcus aureus COL Growth Curve



S. Aureus COL Minimal Bactericidal Concentration (MBC) Results

COL				(*~500ugG/AA)		
Hour	GC=Growth Control			*DMSO GC		
	10 ⁰	10 ⁻²	10 ⁻⁴	10 ⁰	10 ⁻²	10 ⁻⁴
4	TNTC	TNTC	57	TNTC	TNTC	62
5	TNTC	TNTC	76	TNTC	TNTC	73
6	TNTC	TNTC	90	TNTC	TNTC	100
7	TNTC	TNTC	100	TNTC	TNTC	75
8	TNTC	TNTC	80	TNTC	TNTC	102
24	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC

COL				COL		
Hour	500ug G/AA			250ug G/AA		
	10 ⁰	10 ⁻²	10 ⁻⁴	10 ⁰	10 ⁻²	10 ⁻⁴
4	TNTC	28	1	0	0	0
5	TNTC	47	1	0	1	0
6	TNTC	50	3	0	0	0
7	TNTC	52	1	0	0	1
8	TNTC	54	1	0	0	1
24	TNTC	TNTC	TNTC	0	TNTC	TNTC

Eradication of Fecal Coliforms in a Bioreactor

Conversion of Cotton Gin Waste and Dairy Cattle Manure to Methane

- ❑ Bioreactor Conversion of Cotton Gin Waste and Manure to Methane
- ❑ Eliminated Fecal Coliforms
- ❑ Gossypol Likely Responsible
- ❑ Repetition Planned
- ❑ NMSU Horticulture Department Grew Glanded and Glandless Cotton



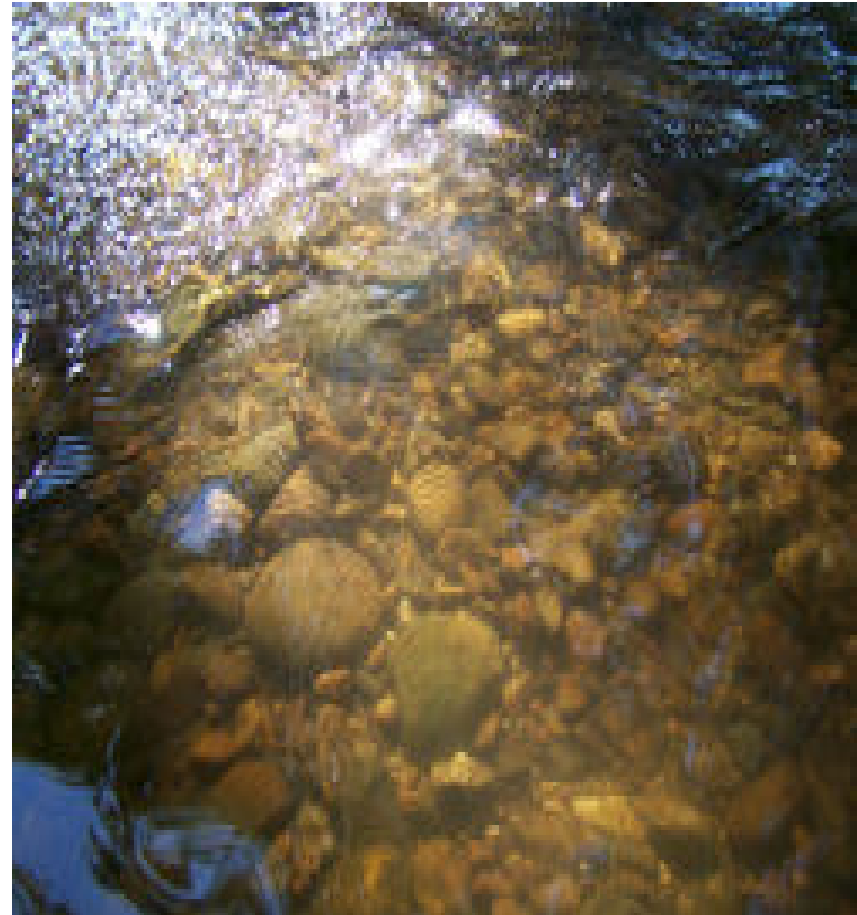
Planned Repetition of Bioreactor Conversion Experiment

- ❑ Glanded versus Glandless Cotton
- ❑ Glandless Cotton Lacks Gossypol
- ❑ Expected Results:
Glanded Will Eradicate All Fecal Coliforms;
Glandless Will Not Eliminate All Fecal Coliforms



Applications for Bioreactor Experiment

- ❑ Utilizes Common Agricultural Wastes
- ❑ Methane Produced for Energy
- ❑ Elimination of Fecal Coliforms
- ❑ Prevention of Water Contamination



Acknowledgements

- ❑ My advisor, Dr. Geoffrey Smith, for his ongoing support of my research.
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- ❑ T.S. Riordan, for conducting the bioreactor experiment that initially sparked my interest in gossypol.
- ❑ My labmates, for sharing our limited lab space and materials with me.

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