SAFETY RECYCLING OF GINNING COTTON WASTES (GIZA90) BY USING THE FUNGI SYSTEM INTO KOJIC ACID AND CELLULASE

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Abstract - This research project deal with biological experiments and methodical development of photochemistry & analytical biochemistry emphasizing the solution of ecological problems affect on people as health hazards and recycling the wastes into economic substance without any ecological harmful effect.

Keywords - Cellulase Enzyme , Cotton , Fungi , Kojic Acid , Recycling , Safety Wastes.

I. INTRODUCTION

Pervious work in the Bacillus licheniformis is a saprophytic bacterium that is widespread in nature and thought to contribute substantially to nutrient cycling due to the diversity of enzymes produced by members of the species. It's used in the fermentation industry for production of proteases, amylases, antibiotics, and specialty chemicals for over a decade with no known reports of adverse effects to human health or the environment [1]. The evidence supporting organic dust as a cause of occupationally-related lung disease substantially increased in the past two decades. The aim of this project was to test an essay, which could be used to detect cytotoxic potential of organic dusts found in working environments in the 2005 [2] . The production of cellulose (filter paper) activity endoglucanase and Glucosidase from Aspergillus Niger on three media in liquid shake culture was compared. The culture filtrate of this organism exhibited relatively highest activity of all three enzymes and extra cellular protein content at 7-day interval during the course of its growth on Czapek-Dox medium supplemented with (w/v)1.0% cellulose. Urea as a nitrogen source and pH5% were found to be optimal for growth and cellulase production by A. Niger. Among various soluble organic carbon sources and line celluloses tested in this study carboxymethyl cellulose and sawdust at 1% supported maximum production of all three enzymes by A. Niger A Modified Method to visualize infection sites of spores of Beauveria bassiana, an Entomopathogen on the Citrus Root Weevil, Diaprepes abbreviatus Beauveria bassiana is a
widespread entomopathogen which is infectious to a great variety of insects. A commercial preparation of this fungus was used to study its potential as a bio control agent of the citrus root weevil, Diaprepes abbreviatus [3]. In 2008. Two papers The first paper published by using the Safety Recycling of Ginning Cotton Wastes (Giza90) by Using the fungi System into Medicinal Substances (Antibiotic) [4]. The second Safety Recycling of Ginning Cotton Wastes (Giza90) by Using the Bacterial System into Medicinal Substances (Antibiotic) [5]. In our research, we will study using the biological system. Safety recycling of ginning cotton wastes (Giza90) by using the fungal system into Kojic Acid and Cellulase Enzyme.

II. PROCEDURE FOR PAPER SUBMISSION

Collection of Ginning Cotton Wastes Samples: Five Samples of ginning cotton Respectively were collected . From EL-Delta company ginning cotton, Sohag,
Egypt. Each sample was put in a sterile polyethylene Bag , sealed and put in another bag which was also Sealed, storage of seeds in double bag containers to Minimize the loss of water content any yet gives Sufficient aeration. Cotton waste samples were kept in a cool place during storage

Determination of Moisture Content: The moisture Content of waste ginning cotton was determined by the Oven method. The wastes of cotton were grounded in an Electric mill and the flour were dried in the oven for 24 Hour at 105ºC then cooled in a desiccator and weighed to constant weight. The moisture content was then Calculated on the oven dry basis.

Determination of Fungi: The dilution plate method Described by Christensen (1963) [6]and employed by Moubasher and his coworkers was used for the estimation of seeds borne fungi as follows:-

(A) 10 g of each sample was commended for1.5min in 500 ml of 0.12% sterile plain agar.
(B) (B) 2 ml of the suspension was transferred using Sterile Menzies's (1959) [7]dip through a known volume Of the suspension.
(C) 1ml of the desired dilution was transferred aseptically into each of five sterile Petri-dishes and 15-
(D) 20ml of an appropriate agar medium, could just Above the solidification temperature, were added to each dish.The dishes were rotated by hand in a broad Swirling motion to disperse the dilution of wastes Ginning cotton in the agar medium.

(E) The dishes were incubated at 28 ºC ±2 ºC for 7-10 days during which colonies were counted,

Isolated and identified. The average number of colonies Per dish was multiplied by the dilution factor to obtain the Number per mg in the original sample. One medium

Was used for isolation The Glucose Czapek's agar Medium:-
(1) 1000 ml.Distilled water.
(2) 10GM glucose.
(3) 2 GM NaNo3
(4) 1 GM KH2P04
(5) 0.5GM MgSO4.7H2O
(6) 0.5 GM KCL
(7) Agar agar15GM per liter H2O was used for Solidification the medium.

Under Mesophilic Temperature 28°C the following were isolated as the followers

Sample (A): Rhizopus stolonifer grows on silicon dust Sample (B): Rhizopus stolonifer and Cladosporium cladosporioides grows on Treatment dust
Sample (C): Rhizopus stolonifer, Aspergillus Niger
Sample (D): Aspergillus Niger, Cladosporium and Aspergillus flavus grow on maxed of wastes
Cladosporioides and Fusarium oxysporum grows in the residues seed of wastes
Sample (E): Residues cellulite cotton of wastes grows nothing
Under Thermophilic temperatures 50 °C
Sample (A): Pencillium duponti grows on silicon dust
Sample (B): Treatment dust grows nothing
Sample (C): Aspergillus fumigatus grows on maxed of wastes
Sample (D): Residues seed of wastes grows nothing
Sample (E): Residues cellulite cotton of wastes grows nothing

Under temperature pysophilic (0 °C - 15°C) hadn't anything at all the samples of the wastes

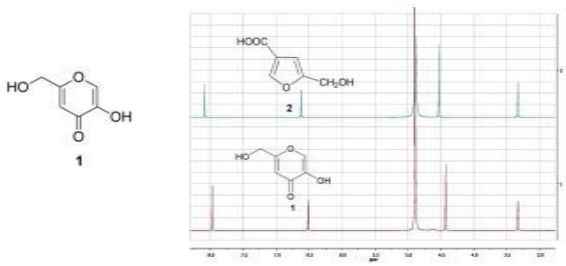
The isolation of cellulite fungi, which can grow variously on cellulose cotton wastes

Under mesophilic temperature were Rhizopus stolonifer, Aspergillus Niger, Aspergillus flavus and Cladosporium cladosporioides where under Thermophilic temperature grow Aspergillus fumigatus and Pencillium duponti.

Table 1. The samples are isolated and identified As follows;

Samples	(Mesophilic fungi) 37 ºC	(Thermophilic fungi) 50 ºC
Sample (A) Silicon Dust.	Rhizopus stolonifer	Penicillin duponti
Sample (B) Treatment dust.	Rhizopus stolonifer and Cladosporium cladosporioides	Nothing
Sample (C) Maxed of wastes.	Rhizopus stolonifer, Aspergillus niger and Aspergillus flavus	Aspergillus fumigatus
Sample (D) Residues of seeds of wastes	Aspergillus Niger, Cladosporium Cladosporioides and Fusarium oxysporum	Nothing
Sample (E) Residues cellulite cotton of wastes	Nothing	Nothing

Aspergillus Niger had the ability to produce Kojic Acid. [8] (Fig. 17) and Cellulase Enzyme (Fig. 18)
Table 2. 1H-NMR (500 MHz) dan 13C-NMR (125 MHz) Data
for kojic acid [9].



1 H-NMR	13 C-NMR	1H-NMR 2
	170.41	
6.49 s, 1H	110.74	
	176.86	
	147.37	
	141.0	
	61.18	

Cellulose Enzyme assay
Microbial Strain
The fungal culture Aspergillus Niger used in the present study was isolated.

Fungus Cultivation for Spore Production and Inoculums Preparation
The fungal inoculum density of 2x106 Spores were used for cellulose production by submerged fermentation method and quantification was carried out by enzyme assays such as Filter paper Endoglucanase and β-D-Glucosidase

Determination of Fungal Biomass
The fungal biomass were estimated by incubating the fungal culture for 7 days. After incubation, the

contents of the flasks were aseptically passed through a pre-weighed filter paper (Whatman No.1) to separate mycelial mat from culture filtrate. The filter paper along with mycelial mat was dried at 70°C in an oven until constant weight and the weight was recorded. Difference between the weights of the filter paper bearing mycelial mat and weight of
pre-weighed filter paper represented fungal biomass, which was expressed in terms of dry weight of mycelial mat (mg/100 ml of CzapekDox medium)

DETERMINATION OF CELLULASE ACTIVITY

The filtrates obtained after removal of mycelial mat by filtration through filter paper was used as an enzyme source. Flasks containing the growing culture of Aspergillus Niger were withdrawn at every 7- day interval for processing.

Cellulose [(filter papers (FPase) and carboxymethyl cellulose (CMCase)] activities was defined as the amount of enzyme which releases 1µmole of reducing sugars per min with glucose as standard under the assay condition .

The values of enzymatic activity were expressed as U/ml for SmF and U/g of dry my cell bran (DMB) for SSF

A series of experiments were carried out to determine the factors for enhanced production of cellulose by Aspergillus Niger [10].



Fig. 1 Sample (A) Silicon dust



Fig. 2 Sample (B) Treatment dust



Fig. 3 Sample (C) Maxed of wastes



Fig. 4 Sample (D) Residues seeds of wastes



Fig. 5 Sample (E) Residues cellulite cotton of wastes



Fig.6 Aspergillus flavus



Fig. 7 Cladosporium cladosporioides

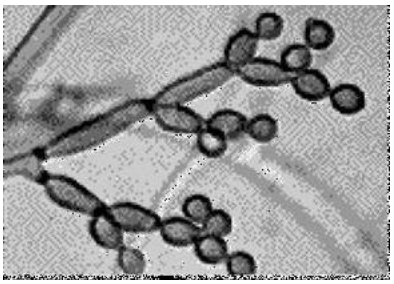


Fig. 8 Cladosporium cladosporioides



Fig. 9 Aspergillus flavus

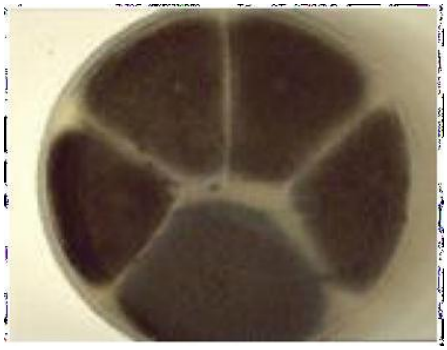


Fig. 10 Cladosporium cladosporioides

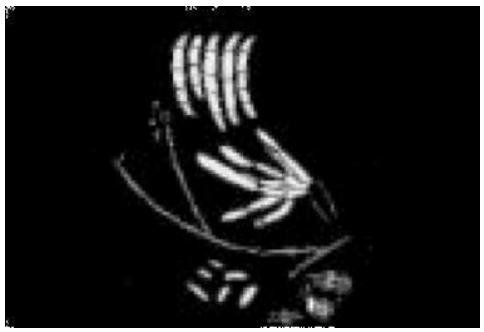


Fig. 11 Fusarium oxysporum



Fig. 12 Aspergillus fumigates



Fig. 13 Aspergillus fumigates

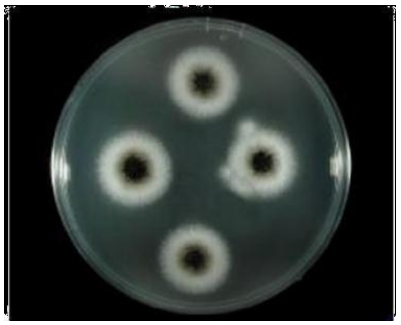


Fig. 14 Aspergillus Niger

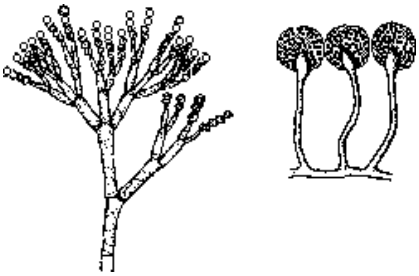


Fig. 15 Pencillium duponti



Fig. 16 Rhizopus stolonifer

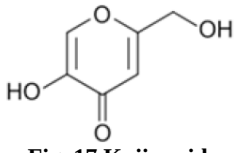


Fig. 17 Kojic acid

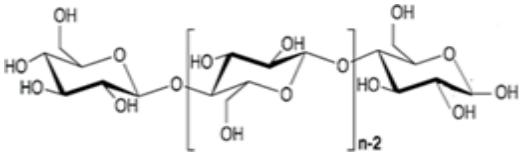


Fig. 18 Cellulase Enzyme

III. CONCLUSION

Microbial sources of cellulose enzyme and Kojic acid are of highly great importance in the current economic value due to their versatile industrial and commercial applications.

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