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Bio-based polysaccharide fungal polymers production from wastes and its antiviral activity against Herpes simplex virus

Mai M. Elhateir[a*], Seham F. Hasan[a], Nagwa M. Sidkey[a] and Mostafa M. Abo Elsoud[b]

[a] Botany and Microbiology department- Al- Azhar University (Girls Branch), Egypt
[b] Microbial Biotechnology Department, National research Centre, Dokki, Giza, Egypt

Abstract

Biobased- polysaccharide polymers were synthesized using two different harsh environments the inhabitant (oil based medium and high salt medium) fungal isolates. They have shown 99.5% identity of 98% query to *Aspergillus terreus* and 99.37% identity of 99% query to *Penicillium griseofulvum* strain P-1707, respectively on genetic identification. They were given the name and code *Aspergillus terreus, f1 and Penicillium griseofulvum, f2,* respectively. Both isolates exhibited strong affinity of growth on different wastes with the highest biopolymers productivity (1.25 & 1.27 g/l) while using the sesame husk (Sem) as the starting raw material. Statistical optimization using Placket-Burman design increased biopolymers yield up to 2.74 & 2.13g/l, respectively at dipotassium hydrogen phosphate (K$_2$HPO$_4$), 2.77 g/l; magnesium sulphate heptahydrate (MgSO$_4$.7H$_2$O), 1.421 g/l; Yeast Extract, *3;* Sesame husk, 57.3; Inoculum Size, 44.66% ; Initial pH 5, Incubation Temperature 50 ^{O}C and Incubation period 4.2 days, While f2 at K$_2$HPO$_4$, 3 g/l; MgSO$_4$.7H$_2$O, 2 g/l; Yeast Extract, *5;* Sesame husk, 95.42 g/l; Inoculum Size, 1%; Initial pH 8, Incubation Temperature 37.5 ^{O}C and Incubation period 6 days. The biopolymers were purified using the ethanol precipitation method. The finally selected two biopolymers were characterized using appropriate techniques including FTIR, 1H NMR and viscosity measurements confirmed their polysaccharide nature. The purified polysaccharide biopolymers were applied on Herpes simplex type 1virus (HSV-1) cells at maximum non-cytotoxic conc. (MNCC) developed from cytotoxicity measurements giving a moderate antiviral activity with EC$_{50}$ of 94.23±2.65 and157.98±3.52. These biopolymers represented a promising environmentally benign antiviral therapy against HSV-1.

Key words: Aspergillus terreus; Antiviral activity; Biobased-polymers; statistical optimization; Herpes simplex type 1virus; Penicillium griseofulvum and wastes

1. Introduction

The herpes simplex virus is responsible for about 75% of oral infections rather than its detrimental effects on immunocompromised people. The herpes simplex virus is struggling with increasing morbidity and mortality in immunocompromised human beings.

It is not an easily treatable virus, in other words, it can be called Feisty Virus. Its common problem is its latency establishment in the host, meaning its long-term existence of the viral genome without detecting infections with the capacity to reactivate under specific conditions. [1-3]. The virus is transferred primarily through direct contact with a lesion or with infected body fluids and an asymptomatic person represents a virus carrier that can increase its dispersal [4].

Numerous publications have explored the antiviral activity of biopolymeric compounds, mainly against human norovirus (HuNoVs) hepatitis A virus (HAV) and coronavirus as food disease problems causing enteric viruses [5-7].

Polymeric compounds are of great interest due to their wide field of applications. Bio-based polymers were produced by bacteria, fungi and plants [8-15]. Biopolymers can be used as emulsifiers to improve the stability, maintenance of physicochemical properties and preservation of formulated drugs, food and cosmetic products [16-17]. Biopolymers (BPs) are the most frequently used nanocarriers for drug delivery as they are biodegradable, compatible with human tissues and body fluids and easy to manipulate in the laboratory.

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Also, biopolymer films have been regarded as potential replacements for synthetic films in food packaging applications representing eco-environmentally materials [18-19].

Biopolymers are compounds consisting of repeating monomers comprising a large group of chemically essential materials produced by living organisms. However, polymers that are synthesized chemically by humans from biological sources such as vegetable oils, fats, resins, sugars, proteins, and amino acids can also be called biopolymers [20]. Based on structure, biopolymers can be divided into; proteins (polymers of amino acids), genetic material (polymers of nucleic acids), glycoforms (carbohydrates and glycosylated molecules), metabolites, polysaccharides and other structural molecules [21-22].

Depending on the origin of the biopolymers, biopolymers can be categorized into four groups including natural biopolymers extracted from biomass (e.g., agro-resources), synthetic biopolymers from microbial production or fermentation (e.g., PHA), synthetic biopolymers i.e. chemically synthesized from biomass (e.g., PLA) and synthetic biopolymers that are chemically synthesized from petroleum products [23].

Biopolymers have unique chemical and physical properties that prefer their use rather than chemically synthesized traditional polymers such as higher viscosity and gelling power, applicable functioning under a wide range of pH, temperature and stability in high ion concentrations. The advantages of the biopolymers production process are avoiding the high costs and minimizing the pollution from other pathways for its synthesis and extraction [24].

This research challenges were the production of novel biopolymers from microbiological entities (fungi) using waste material as the initial raw substrate to be used as an antiviral agent against Herpes simplex virus comprising low-cost efficient manufacturing process.

2. Experimental

2.1. Isolation

Two fungal isolates (f1 and f2) were obtained from two different harsh environments (oil-based and high salt media), respectively in previous studies. Concerning the first isolate a medium composed of (g/l) NaNO$_3$, 2; K$_2$HPO$_4$, 0.5; MgSO$_4$.7H$_2$O, 0.5 with the addition of 1% oil as a carbon source was used, after mixing, it was sterilized at 121°C at 1.5 atm for 20 min, then poured into sterilized Petri dishes followed by inoculation with 0.1 ml of used engine oil waste obtained from Nasr city, Cairo, Egypt. Incubation was at 28°C for 7 days. After that, the

high growth isolates were picked and purified using the same protocol.

The second isolate was gained through the following medium: MgSO$_4$.7H$_2$O, 0.1; K$_2$HPO$_4$, 3.12; KH$_2$PO$_4$, 0.28; L-Glutamine, 4.97; Glucose, 10; Agar-Agar, 20 with the addition of NaCl, 4%. Inoculation was done by adding 0.1 ml of industrial waste soil obtained from a detergent factory in Alameria area, Cairo, Egypt. Incubation was at 30 ^{O}C for 5 days followed by purification under the same conditions.

2.2. Cultivation and identification of biopolymers producing isolates

The highly growth isolates were kept on C'zapeck Dox medium, and then the finally selected isolates were subjected to genetic identification. First, DNA extraction was conducted using DNeasy kit (Qiagen), then the DNA was passed to agarose gel electrophoresis step in which the DNA was visualized and imaged using the transilluminator of a gel documentation system (BIO-RAD, Gel Doc 2000). *Taq* PCR Master mix (purchased from Qiagene) was used to amplify or synthesize DNA (genomic or plasmid) fragments using a thermal cycler machine (gradient Robocycler 96 Stratagene, USA) at the Regional Center for Mycology and Biotechnology. The interested DNA fragments (PCR products and linear plasmid) were purified by agarose gel electrophoresis, after that sequencing of plasmid and amplified PCR fragments was carried out by Cy5/Cy5.5 Dye Primer Sequencing kit from Visible Genetics Inc. for use with the Open Gene automated DNA sequencing system [25-26] at the Regional Center For Mycology and Biotechnology. Primer sequences used for the identification of 18s in the current study were

ITS1 (5'- TCC GTA GGT GAA CCT GCG G-'3)
ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-'3)

2.3. Screening the growth ability of f1 and f2 on different wastes

The medium used consists of (g/L) NaNO$_3$, 3.0; K$_2$HPO$_4$·3H$_2$O, 1.3; Citric acid, 0.7; MgSO$_4$·7H$_2$O, 0.5; KCl, 0.5; FeSO$_4$. 7H$_2$O, 0.05; Yeast extract, 1.0 with the addition of 5% for each waste type (Sesame husks (Sem), Molasses (M), Corn syrup (Co), Rice water (RW), Bagasse (Ba), Sawdust (Sd) and Water hyacinth (Wh), separately as a carbon source and Agar; 20. The same medium with replacing the waste with glucose, 30 and sucrose, 20 was regarded as

positive control; pH was adjusted at 4.5. Incubation was carried out at 30 ℃ for 7 days.

2.4. Production of biopolymers

The medium used for production of biopolymers contained the same components as that used for screening the growth of both fungal isolates (f1 & f2) on different tested wastes except that the agar was excluded. Each broth culture medium was inoculated with 4 % inoculum of 7 days fungal culture of each strain. The flasks were incubated for 7 days at 30 ℃ on a rotary shaker at 150 rpm.

2.5. Optimization of biopolymers production conditions

The optimum conditions for biopolymers maximum production process was screened using Design Expert, Version 13.0.5.0 Stat-Ease, Design Expert Inc., Minneapolis, 2021.

Herein, eight nutritional and physical factors have been introduced constructing Placket Burman design consisted of 15 runs for studying their effect on biopolymers production by both fungal isolates (f1 & f2), measuring biopolymers production as a response in g/l (**Table 1**).

2.6. Validation

The resulted model was validated practically and compared to the predicted numerical optimization results for maximum biopolymers production.

2.7. Purification and recovery of exo-biopolymer

The broth culture media were centrifuged at 5000 rpm for 30 min followed by filtration to remove any residual fungal mycelia pellets. The supernatants were diluted (1:1) with ethanol 95% for precipitation of the produced biopolymers. The diluted mixture was kept overnight at 4℃. Then, the mixture was centrifuged at 4000 rpm for 10-20 min. for collection of the precipitated biopolymers. The precipitates were dried at 60℃ overnight. The weight of each precipitated polymer was determined. The primary structure of the highest-produced biopolymers was subjected to analysis using FTIR and H1 NMR.

2.8. Viscosity determination of produced biopolymers

Viscometry or viscosity method was one of the first methods used as an indication determinant for the molecular weight of polymers. The simplest method used is capillary viscometry by using the Ostwald viscometer. The relative viscosity of biopolymers was measured by recording both the flow time of the polymeric solution (t) and the flow time of the pure solvent (t_0). The ratio of the polymer solution flow time (t) to the flow time of pure solvent (t_0) is equal to the ratio of their viscosities (η/η_0) only if they have the same densities [27].

2.9. Antiviral activity of produced biopolymers against Herpes simplex type 1 virus (HSV-1)

Vero cells (derived from the kidney of African green monkey) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The Vero cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, HEPES buffer and 50 μg/mL gentamycin. All cells were maintained at 37℃ in a humidified atmosphere with 5% CO_2 and were subcultured two times a week [28]. The cytopathogenic Herpes simplex type 1 virus (HSV-1) was propagated and assayed in confluent Vero cells [29]. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose (TCID50) with eight wells per dilution and 20μl of inoculum per well using the Spearman-Karber method [30]. The antiviral screening was performed using a cytopathic effect inhibition assay. This assay was selected to show specific inhibition of a biological function, that is, a cytopathic effect in susceptible mammalian cells measured by the MTT method against Herpes simplex type 1 virus (HSV-1) [31-32].

Table 1

Summary of the factors used in Placket Burman design using f1 &f2 for biopolymers production

Factor	Name	Factor level (g/l biopolymers)	
		Lower Limit	Upper Limit
A	$K_2HPO_4.3H_2O$	1	3
B	$MgSO_4.7H_2O$	1	3
C	Yeast Extract	1	5
D	Sesame husk	10	100
E	Inoculum Size	1	5
F	Incubation Temperature	25	50
G	Initial pH	4	8
H	Incubation period	4	8

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3. Results

Two fungal isolates f1 & f2 (oil-degrading and salt tolerant, respectively) were selected from previous studies and identified genetically for examination of their biopolymers production ability. They have shown 99.5% identity of 98% query to *Aspergillus terreus* and 99.37% identity of 99% query to *Penicillium griseofulvum* strain, respectively. They were given the name and code as *Aspergillus terreus,* f1 *and Penicillium griseofulvum* P-1707, f2, respectively (**Fig. 1, 2 and 3**).

Herein, seven different wastes (Sesame husks (Se), Molasses (M), Corn syrup (Co), Rice water (RW), Sawdust (Sd) and Water hyacinth (Wh)) were used as the starting raw material for the production of biopolymers using *Aspergillus terreus,* f1 & *Penicillium griseofulvum* P-1707, f2. The growth ability of both fungal strains was monitored on the seven mentioned substrates with comparison to Glucose+Sucrose (GS) as a positive synthetic starting substrate (**Table 2**). The highest growth was observed when using Sesame husks (Se) followed by Molasses (M), Corn syrup (Co) and Glucose+Sucrose (GS). Quantification of the produced biopolymers was performed by applying the finally selected wastes revealed that, the highest biopolymers productivity (1.25&1.27) was achieved while using Sesame husks

(Se) compared to Glucose+Sucrose (GS) with weight (1.01&1.09) for f1 and f2 , respectively (**Table 3 and Fig 4**).
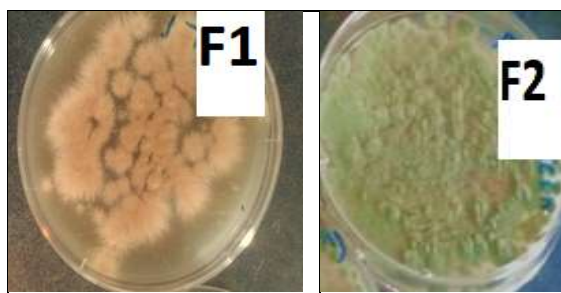

Fig.1. Growth of f1and f2 biopolymers producing isolates on C'zapeck Dox medium

Fig.4. Purified biopolymer precipitates by f2 and f1 using Sesame husks (Se) (1,2), respectively.

Table 2

Screening the growth ability of *Aspergillus terreus,* f1 and *Penicillium griseofulvum* P-1707, f2 on different wastes based media

Strain used	Growth on different wastes							
	Sesame husks (Se)	Molasses (M)	Corn syrup (Co)	Rice water (RW)	Bagasse (Ba)	Sawdust (Sd)	Water hyacinth (Wh)	Glucose+ Sucrose (GS)
Aspergillus terreus, **f1**	++++	++++	+++	+	+++	-ve	+	+++
Penicillium griseofulvum **P-1707, f2**	++++	++++	+++	+	++	+	+	+++

where; ++++ means heavy growth, +++ good growth, ++: moderate growth, +: weak growth and –ve: no growth

Table 3

Different polymers weight on the best selected wastes

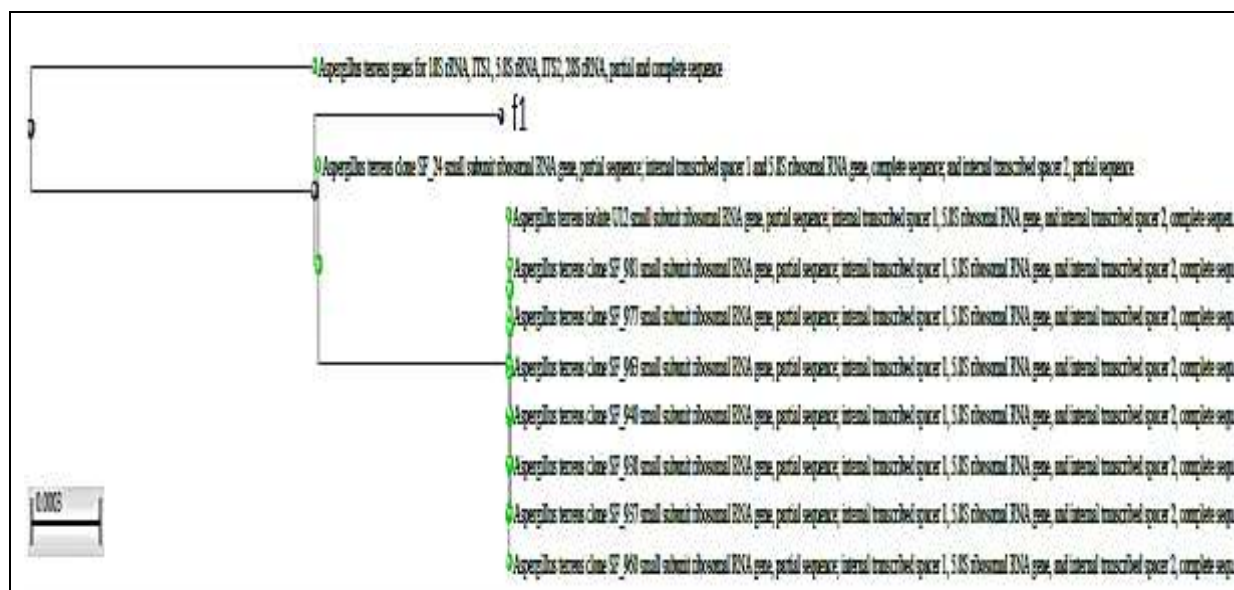| Strain used	Weight of produced biopolymer (g/l)			
	Sesame husks (Se)	Molasses (M)	Corn syrup (Co)	Glucose+Sucrose (GS)
Aspergillus terreus, **f1**	1.25	1.20	1.12	1.01
Penicillium griseofulvum **P-1707, f2**	1.27	0.53	1.25	1.09

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Fig. 2. A dendrogram showing the sequence relationships between f1 and several other related strains
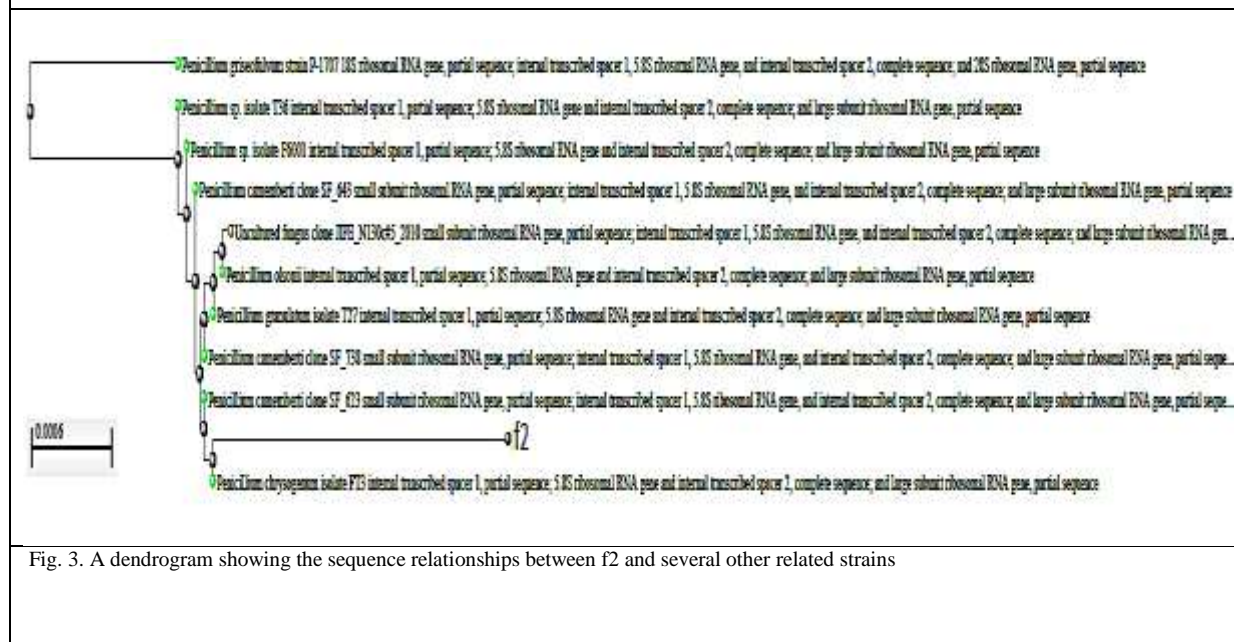

Fig. 3. A dendrogram showing the sequence relationships between f2 and several other related strains

Analytical studies for exploring the possible structures of the produced biopolymers were performed using FTIR and 1H NMR **(Fig. 5, 6, 7 & 8)**. The FTIR spectrum of the biopolymer produced by *Aspergillus terreus*, f1 has showed peaks at 3625, 3685 cm^{-1} corresponded to N-H; 3416 cm^{-1} broad band corresponded to OH, 2852 and 2921 cm^{-1} corresponded to sp3 H (-CH3), a characteristic absorption band appeared at 1744cm^{-1} assigned to the stretching vibration C=O characteristic of the band amide I**;** while 1004 cm^{-1} medium band correspond to C-N**.** Also, FTIR spectrum of the biopolymer

produced by *Penicillium griseofulvum* P-1707, f2 has showed peaks at 3416 cm^{-1} broad band corresponded to OH; 2929 cm^{-1} corresponded to sp3 H (-CH3); sharp strong band at 1645 cm^{-1} correspond to the stretching vibration C=O characteristic of the band amide I, while 1078 cm^{-1} medium band correspond to C-N **[33-34]**.

Both biopolymers have exhibited polysaccharide structure, the infrared spectra of the biopolymers showed the presence of hydroxyl, amine and C-N bonds for samples 1 and 2 which was confirmed by NMR 1H analysis spectrum of sem, *Aspergillus*

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terreus, f1 biopolymer which showed peaks at 1.0431, 1.0605, 1.0780, 1.1684, 1.1969, 1.2150 and 1.2378 ppm corresponding to H of a methyl group –CH_3 and methylene groups -CH_2. Peaks at 3.3928, 3.4190, 3.4371, 3.4546 and 3.4719 ppm were corresponding to different H of–CH. The peak at 4.3766, 5.2033 and 5.3211 ppm corresponded to H_2 of amino group –NH_2. The 1H NMR spectrum of sem, *Penicillium*

griseofulvum P-1707, f2 biopolymer showed peaks at 1.0435, 1.0610 and 1.0784 ppm corresponding to H of methyl group –CH_3 and methylene groups -CH_2. Peaks at 3.3821, 3.4196, 3.4373 and 3.4548 ppm were corresponding to different H of–CH while; 4.3766 ppm corresponded to H_2 of the amino group –NH_2.
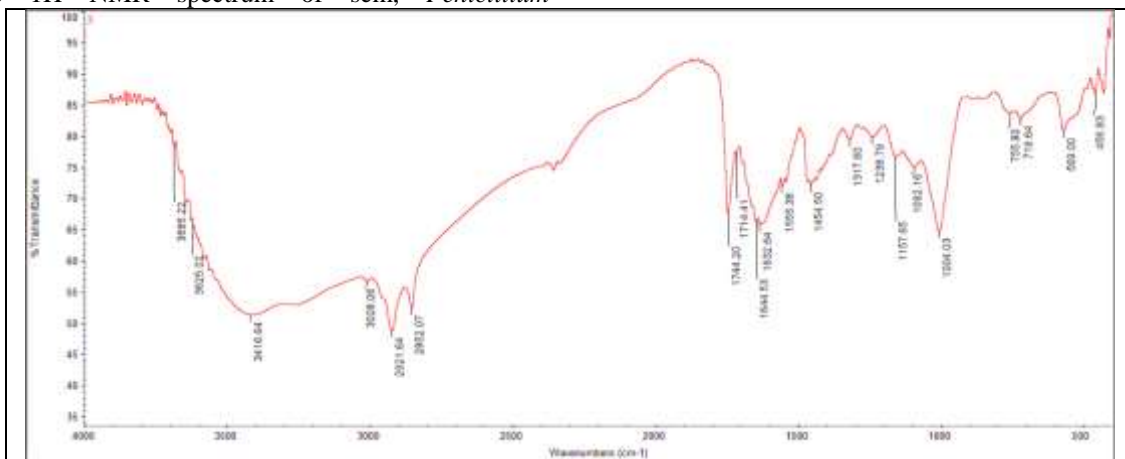

Fig. 5. FTIR spectrum of biopolymer produced *Aspergillus terreus*, f1
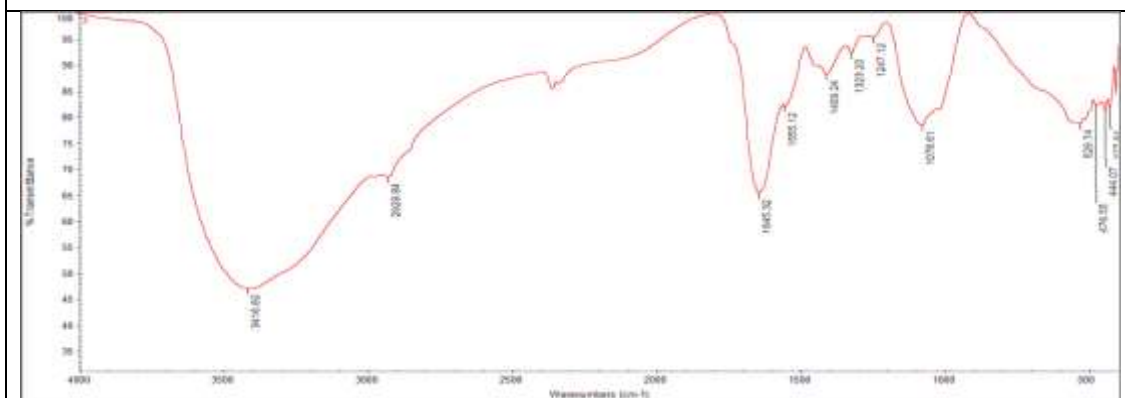

Fig. 6. FTIR spectrum of biopolymer produced by *Penicillium griseofulvum* P-1707, f2
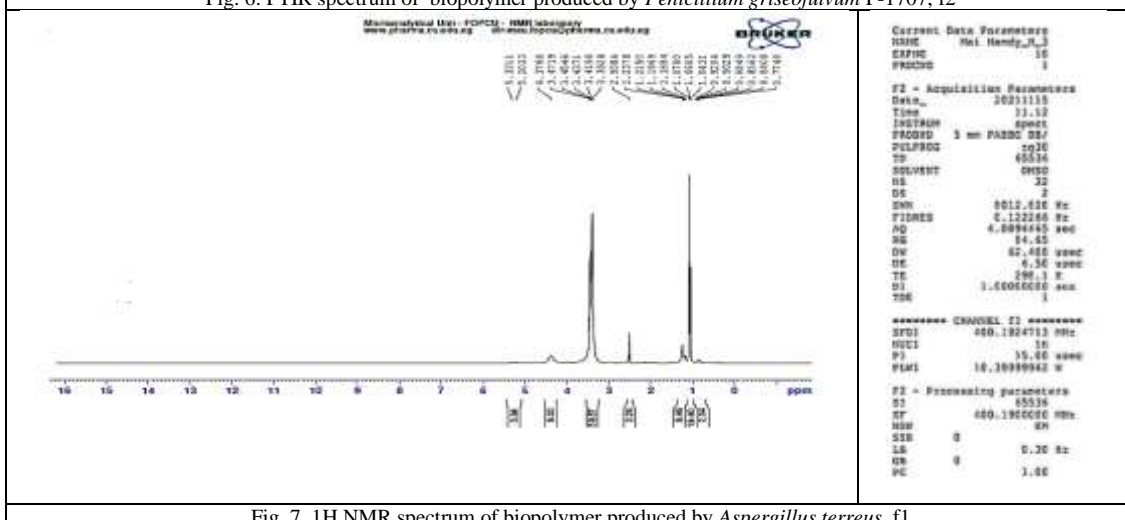

Fig. 7. 1H NMR spectrum of biopolymer produced by *Aspergillus terreus*, f1
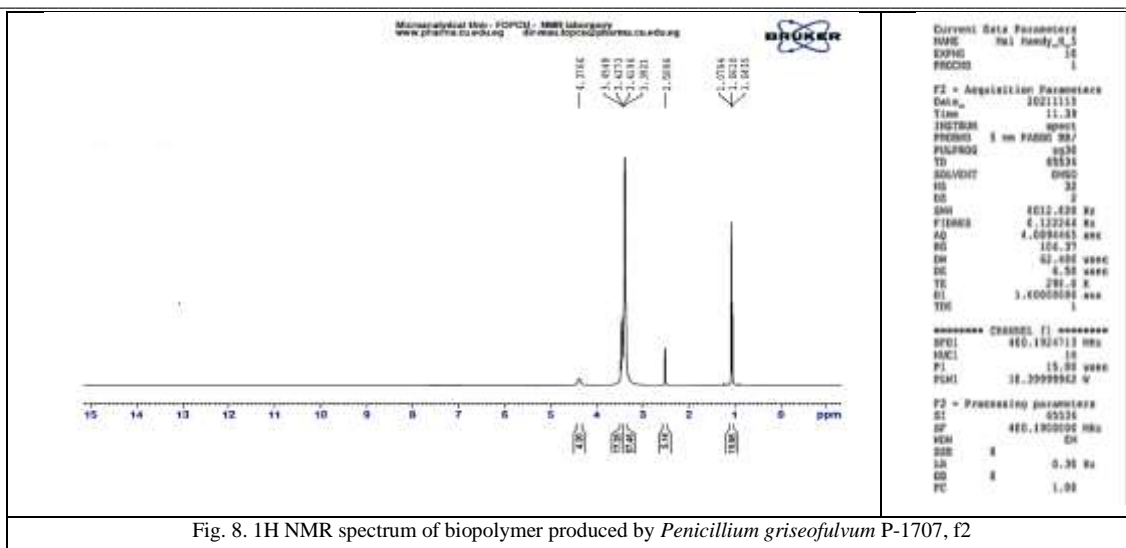
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Fig. 8. 1H NMR spectrum of biopolymer produced by *Penicillium griseofulvum* P-1707, f2

3.1. Statistical optimization for biopolymers production was done as follows:

Eight nutritional and physical factors viz. (K_2HPO_4, $MgSO_4.7H_2O$, Sesame husk, Yeast extract, inoculum size, incubation temperature, initial pH and incubation period) have been screened using Placket-Burman design monitoring their effect on biopolymers production by *Aspergillus terreus*, f1 and *Penicillium griseofulvum* P-1707.

The provided data in **Table (4)** concerning biopolymers production by *Aspergillus terreus*, f1 illustrated that, the model and model terms are significant as model F-value of 44.19, Prob > F less than 0.05 and A, B, D, E, F, G, H, AD are significant model terms. In addition, the lack of fit F-value of 0.32 implies the lack of fit is not significant relative to the pure error which was a good indication.

Also, the results indicated a good correlation between actual and predicted results with (R2 = 0.99) with adequate Precision of 21.4834 the model can be used to navigate the design space **(figure 9a)**.

Table 4

Analysis of Variance (ANOVA) of Plackett - Burman design results for biopolymer production by *Aspergillus terreus*, f1

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.43	8	0.5532	44.19	0.0003	significant
A-K_2HPO_4	1.38	1	1.38	110.59	0.0001	
B-$MgSO_4.7H_2O$	0.7410	1	0.7410	59.19	0.0006	
D-Sesame husk	0.1643	1	0.1643	13.12	0.0152	
E-Inoculum Size	1.56	1	1.56	124.64	0.0001	
F-Incubation Temperature	0.8630	1	0.8630	68.94	0.0004	
G-Initial pH	0.5189	1	0.5189	41.45	0.0013	
H-Incubation period	0.5770	1	0.5770	46.09	0.0011	
AD	0.5010	1	0.5010	40.02	0.0015	
Curvature	0.2577	1	0.2577	20.58	0.0062	
Residual	0.0626	5	0.0125			
Lack of Fit	0.0204	3	0.0068	0.3233	0.8134	not significant
Pure Error	0.0422	2	0.0211			
Cor Total	4.75	14				

The Final Equation for biopolymer production by *Aspergillus terreus*, f1 using Plackett - Burman design is as follow:

Biopolymer (g/l) = - 0.090139+ 0.714278 K_2HPO_4 - 0.277833 $MgSO_4.7H_2O$ + 0.016222 Sesame husk + 0.201583 Inoculum Size + 0.029983 Incubation Temperature - 0.116250 Initial pH - 0.122583 Incubation period - 0.006811 K_2HPO_4 * Sesame husk.

Data in **table (5)** concerning biopolymers production by *Penicillium griseofulvum,* f2 indicated that, the model and model terms are significant as model F-value of 9.78, Prob > F less than 0.05 and A, EG are significant model terms. In addition, the lack of fit F-value of 0.20 implies the lack of fit is not significant relative to the pure error which was good indication. Also, the results indicated a good correlation between actual and predicted results with (R2 = 0.92) with adequate precision of 9.88 and the model can be used to navigate the design space **(figure 9b)**.
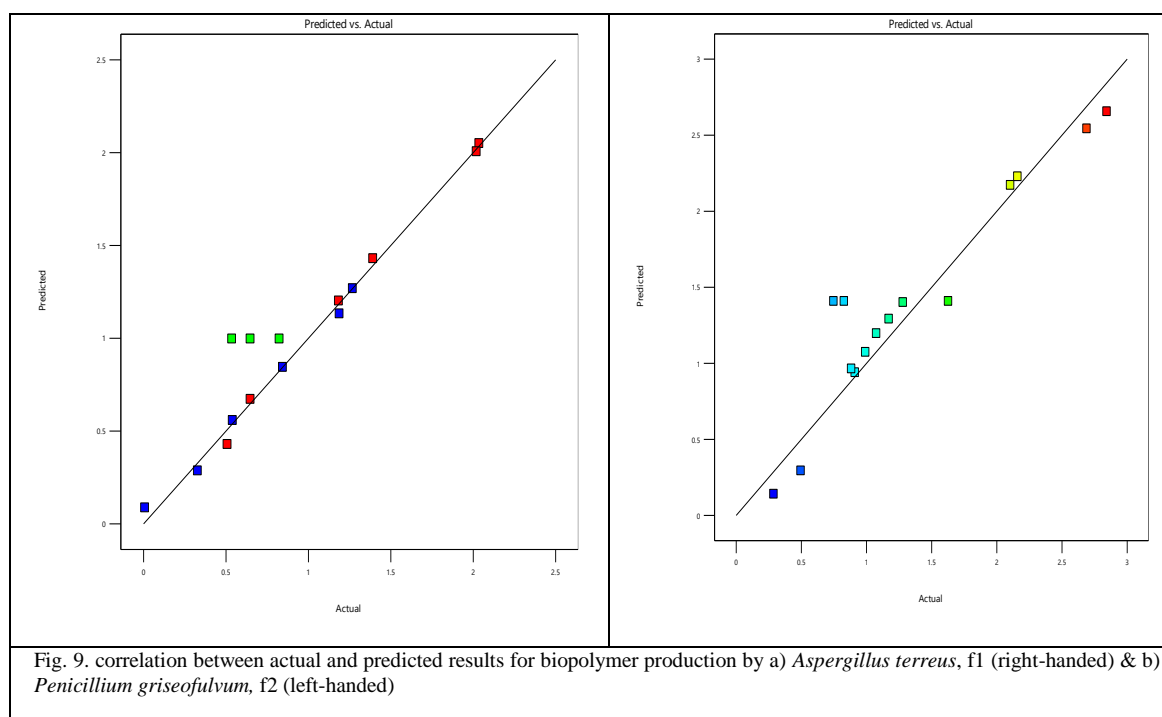

Fig. 9. correlation between actual and predicted results for biopolymer production by a) *Aspergillus terreus*, f1 (right-handed) & b) *Penicillium griseofulvum,* f2 (left-handed)

Table 5

Analysis of Variance (ANOVA) of Plackett - Burman design results for biopolymer production by *Penicillium griseofulvum*, f2

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.53	7	1.08	9.78	0.0065	significant
A-K_2HPO_4	1.21	1	1.21	11.05	0.0159	
C-Yeast Extract	0.0598	1	0.0598	0.5437	0.4887	
D-Sesame husk	0.3652	1	0.3652	3.32	0.1182	
E-Inoculum Size	0.0531	1	0.0531	0.4826	0.5132	
G-Initial pH	0.0766	1	0.0766	0.6969	0.4358	
CE	0.4909	1	0.4909	4.46	0.0791	
EG	0.7500	1	0.7500	6.82	0.0400	
Curvature	0.2777	1	0.2777	2.53	0.1631	
Residual	0.6598	6	0.1100			
Lack of Fit	0.1862	4	0.0466	0.1966	0.9204	not significant
Pure Error	0.4736	2	0.2368			
Cor Total	8.47	14				

The Final Equation for biopolymer production by *Penicillium griseofulvum*, f2 using Plackett - Burman design is as follow:

Biopolymer (g/l) = - 1.43925 + 0.389667 K$_2$HPO$_4$ + 0.235521 Yeast Extract + 0.004748 Sesame husk + 0.650937 Inoculum Size + 0.200021 Initial pH - 0.065687   Yeast Extract * Inoculum Size - 0.081187 Inoculum Size * Initial pH

3.2. Validation

Based on the suggested solutions obtained by numerical optimization for maximum biopolymer production (2.21 & 2.6 g/l) yield was obtained for *Aspergillus terreus*, f1 & *Penicillium griseofulvum* P-1707, f2 ,respectively at K$_2$HPO$_4$,   2.77 g/l; MgSO$_4$.7H$_2$O, 1.421 g/l; Yeast Extract, *3;* Sesame husk, 57.3; Inoculum Size, 44.66% Initial pH 5, Incubation Temperature 50 ^{O}C and Incubation period 4.2 days, While f2 at K$_2$HPO$_4$, 3 g/l; MgSO$_4$.7H$_2$O, 2 g/l; Yeast Extract, *5;* Sesame husk, 95.42 g/l; Inoculum Size, 1%; Initial pH 8, Incubation Temperature 37.5 ^{O}C and Incubation period 6 days.

The practical results were 2.73 & 2.13 g/l, with a validation percentage of 123 & 81 %, respectively.

Moreover, the viscosity of the produced biopolymers was measured in terms of relative viscosity as an indication for their molecular weights which is an important criterion for identifying good and poor biopolymers **(Table 6)**.

In addition, both purified polymers have been submitted for evaluation of their antiviral activities against Herpes simplex type 1 virus (HSV-1). The results represented in **Table (7)** indicated that, the maximum non-cytotoxic conc. (MNCC) developed from cytotoxicity measurements (15 &50 µg/ml) giving a moderate antiviral activity with EC$_{50}$ of 94.23± 2.65 and 157.98± 3.52 for polymers produced by *Aspergillus terreus,* f1 and *Penicillium griseofulvum* P-1707, f2, respectively depending on inhibition of a biological function of HSV-1, that is, a cytopathic effect in susceptible mammalian cells measured by MTT method.

Table 6

viscosity measurements of the selected biopolymers

sample	Biopolymer relative viscosity
Sem, *Aspergillus terreus*, f1	1.57
Sem, *Penicillium griseofulvum* P-1707, f2	1.37

Table 7

The antiviral effects of the tested compound against Herpes simplex type 1 virus when tested at maximum noncytotoxic conc. (MNCC)

Sample	MNCC (µg/ml)	Antiviral effect on HSV-1 virus (%)	Antiviral Efficiency		
			EC$_{50}$	CC$_{50}$	SI
1	15	22.67±1.39	**94.23±2.65**	**106.81± 3.05**	**1.13**
2	50	17.98±1.91	**157.98±3.52**	**218.64± 5.12**	**1.38**

4.   Discussion

There are enormous amounts of wastes that are produced and accumulated over time through agriculture or industry processing including fruits peels, molasses, bagasse and husks and rice straw that cause serious hazardous problems to the environment **[35]**.

However, several industrial and agricultural wastes are rich in lots of important compounds including proteins, polysaccharides, lipids and minerals needed for the growth of microorganisms. This led to increasing research studies on the structural composition of different waste types to be used as the starting substrates for the production of valuable economically needed compounds **[36-40]**.
Among the trendy needed materials biopolymers due to the unique characteristics that differentiate them from other chemically synthesized polymers such as higher

viscosity and gelling power, stability under a wide range of pH and temperature, stability in high ion concentrations and biodegradability **[41]**.

Herein, both identified fungal strains have shown variable activity of biopolymers production with the maximum obtained while using sesame husk. It was the most suitable substrate for *Aspergillus terreus,* f1 *and Penicillium griseofulvum* P-1707, f2 biopolymer production indicated by the high weight obtained. Structural studies on Sesame husk composition revealed that, it contained proteins, lipids, carbohydrates, fibers and metals such as calcium, sodium, phosphorous, potassium, iron, manganese and zinc that may be contributed to growth enhancement and biopolymers production **[42].**

Most of studies regarding biopolymers production were depending on using bacterial strains as the producers while, a few studies discussed and

approved the capability of fungi as efficient manufacturing biopolymers tools **[43-49]**. It should be mentioned that, fungi have more defined mechanisms for adaptation and growth under stress and nutrient limitation conditions which minimizes the need for the preparation of specific synthetic media for biopolymers production along with the production of novel compounds **[50-53]**.

In this study, The structure of the produced biopolymers semf1 and sem f2 are closely related indicated by FTIR and 1HNMR analysis, both biopolymers having polysaccharide nature as it has shown a similar pattern of FTIR; further structural analysis is recommended for getting more confirmative information and molecular identification of the produced biopolymers.

In addition, The produced biopolymers have shown antiviral activity against HSV-1revealing that they interfered with one of the different stages of viral infection processes (entry, attachment or replicating cycles) indicated by cytopathic effect monitoring through an interaction of these polyanionic compounds with the positively charged domains of the viral envelope glycoproteins **[54]**.

In this respect, different structural biopolymers were produced microbially offering antiviral activity against HSV-1 having protein structure as bacterial polymeric silk which was produced by *Bacillus* sp. strain NE. it consists of two proteins, called fibroin and sericin **[7]** or possesses polysaccharide structure that have the advantage of being less toxic to Vero cells and has a certain antiviral effect inhibiting the adsorption and biosynthesis of HSV-1 within a definite concentration range e.g. the extracellular polysaccharides (EPS) produced by *Paecilomyces lilacinus* **[55]**, in addition, several treatments of microbial polysaccharide biopolymers may lead to new derivatives having antiviral enhancement activity as sulfation, phosphorylation, selenization **[56]**. Also, biopolymers conjugated to nanoparticles can exhibit antiviral activity against different viruses including HSV-1 **[57]**.

5. Conclusion

Bio-based polymers are of great interest due to their characteristics that distinguish them from industrial polymers. This study focused on the production of biopolymers by fungal strains using different wastes as the starting substrates highlighting the most acceptable waste source with minimum production process requirements. The study led to a chance of getting rid of undesired accumulating different waste types with productive routes for novel biopolymers obtained having antiviral activity against Herpes simplex virus. Further research would be encouraging for optimization of the process and assessment of the effect of several waste pretreatment protocols on biopolymers microbial productivity by studying the structural details of the produced compounds as the

emerging of novel antiviral materials is an urgent need for competing for the threats of viruses.

6. Conflicts of interest

There are no conflicts to declare

9. References

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