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Optimization of Different Parameters on Lovastatin Production by *Aspergillus niger* I_4 and *Aspergillus terreus* I_8 and its Hypocholesterolemic Effect.

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ABSTRACT

After seven days of incubation, both fungal isolates *A. niger* I_4 and *A. terreus* I_8 produce maximum lovastatin productivity at 30°C when applying an inoculums size of 10^8 spores/ml with (maize stalk, rice husk and wheat straw) under solid state fermentation conditions and at pH 5 and 6 respectively. Type of nitrogen and carbon sources influenced lovastatin production by both the fungal isolates. Addition of Fe^{+2}, Ca^{+2}, Zn^{+2} and Mg^{+2} enhanced the production of lovastatin by the fungal isolates with the three substrates used. As Cu^{+2} had no effect on lovastatin production by *A. niger* I_4 and it reduced the lovastatin production by *A. terreus* I_8. Supplementation of the two vitamins, B_1 (thiamin) and B_6 (pyridoxine) to the fermentation media enhanced the fungal lovastatin biothynthesis. Results of animal studies revealed that the fungal lovastatin from *A. niger* I_4 induced significant decrease in total cholesterol, triglycerides and LDL while increasing HDL level of albino rats.

Keywords: *Aspergillus niger*, *Aspergillus terreus*, lovastatin , Solid State Fermentation, Agricutural wastes, hypocholesterolemia, albino rats.

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INTRODUCTION

Lovastatin was the first fungal secondary metabolite to obtain approval from the US Food and Drug Administration (FDA) in Agust 1987 after successful clinical trails in which lovastatin proved to dramatically reduced LDL with few side effects (Tobert 2003). Lovastatin (Mevanolin, Monakolin K and Mevacor ^{TM}) is a fungal metabolite produced by *Aspergillus terreus*, it is a pharmaceutical important compound in treating hypercholesterolemia because of its potent inhibitory activity toward hydroxy-3- methylglutaryl-coenzyme A (HMG-CoA) reductase which catalyzes the rate limiting step in cholesterol biosynthesis (Lai *et al*., 2005).

Syarifah *et al.;* (2013) and Jahromi *et al*.; (2013) found that *Aspergillus niger* SARI and *A. terreus* were an excellent potential as a lovastatin producer under Solid Stare Fermentation (SSF). Upendra *et al.* (2013) mentioned that FDA approved *A terreus* as a best microorganism to produce lovastatin. Optimization of different process parameters to maximize lovastatin production by fungi such as : temperature , fermentation time, inoculum volume, pH, culture medium composition and nutrient parameters were examined in several studies (Valera *et al*., 2005, Xu *et al.,* 2005, Lee *et al*., 2006, Lai *et al*., 2007, Sayyad and Panda 2007, Jia *et al* ., 2009, Panda *et al.,* 2010). Kusmana *et al.* (2012) studied the effect of lovastatin produced from *Aspergillus flavus* UICC360 on total cholesterol, triglycerides, HDL, LDL level in blood of white rats (*Rattus novergicus* strain Sprague Dawley). This work aims for studying environmental and nutritional requirements for *A. niger* I_4 and *A. terreus* I_8 for lovastatin production and investigating a hypocholesterolemic effect of lovastatin extraction obtained from *A. niger* I_4 on albino rats.

MATERIALS AND METHODS

Microorganisms used: Two fungal isolates, *A. niger* I_4 and *A. terreus* I_8 were isolated and purified on Czapek's and Dox agar and they were detected for lovastatin production.

Production media: Rice husk, wheat straw and maize stalk obtained from fields (Sharkyia governorate, Egypt). The obtained agricultural substrates were dried at 60^{o}C, then grinded and sieve. These substrates used alone at 10 % w/v without any treatment or addition under solid – State Fermentation conditions. One ml of fungal spore suspension (10^6 spores/ ml) of 7 days old slants of each fungal isolate was added to the different substrates (Triplicates were used), and incubated for 7 days at 28^{o}C and pH 7.0. At the end of incubation period, samples were taken and tested for lovastatin production.

A**ssay of Lovastatin:** Lovastatin productivity (mg / gm) was assayed according to Lingappa *et al*.; (2004).

Environmental and Nutritional Requirements of lovastatin Production:

Incubation period

The production medium was prepared as previously mentioned, Inoculum of 106 spores /ml was used and incubation was carried out at 28^{0}Cat for different time intervals.

Incubation Temperature:

Incubation was carried out at different temperatures for 7 days.

Inoculum Size:

Production media was inoculated by different inoculum sizes (spores/ ml) separately. The pH adjusted at 7, temperature at 30^{0}C, incubation period was 7 days for both fungal isolates.

Hydrogen ion concentration (pH) :

Production media was adjusted at different pH using phosphate- citrate buffer.

Different nitrogen sources:

Different organic and inorganic nitrogen sources were supplemented to the production medium with an equivalent amount of nitrogen to that present in 0.3 % (W/V) $NaNo_3$ in Czapek's liquid medium. The control devoid from any nitrogen source. All other factors were carried out as previously mentioned.

Different carbon sources:

Lovastatin production was investigated under the effect of different carbon sources that were added to the production medium. Lovastatin production (mg/gm) was determined as previously mentioned at the end of incubation period. All the previously factors take in consideration for the two tested isolates.

Divalent metals cations:

Each individual divalent metal cations i.e (Fe^{+2}, Ca^{+2}, Zn^{+2}, Mg^{+2} and Cu^{+2}) were incorporated in the production medium at two concentrations 2 and 4 mM /L . The control devoid from any metals cations. All the previous parameters were adjusted as previously mentioned for both isolates.

Vitamin B1 and B6:

Two vitamins, thiamine and pyridoxine (B_1, B_6) were incorporated to the production media with three concentrations (1, 2 and 4) mg/l. Control used without any vitamin addition. All the previously factors take in consideration.

Hypocholesterolemic effect:

Preparation of lovastatin extraction

Fermentation was carried out in the production media. Inoculation with *A. niger* I_4 under the obtained optimum conditions. After seven days incubation, the culture was filtered and pH adjusted at 3. Extraction with ethyl-acetate in the same amount. After evaporatation lovastatin β-open-hydroxy-acid was obtained.

Determination of the dose of lovastatin

According to recommendations of National Cholesterol Education Program (NCEP), permissible dose of lovastatin for humans is 10-80 mg per day. Based on these recommendations and for use in rats, after being converted multiplied by 0.018 (Shaw, *et al.,* 2008) .The treatment used in this experiment for rats was (0.4 mg/day).

Preparation of lovastatin solution

Stock solution made by dissolving 4 mg of lovastatin with 25 ml of 1% CMC.

Experimental animals:

A total number of 30 white male albino rats aged two months and weighing of 180± 5 g were used for this study. The experimental rats were adapted for 14 days to be adjusted to the new environments. Rats were fed and water *ad libitum*.

Experimental design

Group- I: 10 rats without any treatment (control group).

Group-II: 10 rats received 2.5 ml /day of coconut oil orally for eight days for increasing their cholesterol levels; (Zulfiana, 2003) - (hypercholesteolemic group).

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Group-III:- 10 rats received 2.5 ml of coconut oil concurrently with 2.5 ml of lovastatin by a dose of 0.4mg/day orally for 8 days (Tanzawa *et al* ; 1982) (lovastatin treated group).

At the end of the experiment, all rats were sacrificed by cervical decapitation. Blood samples were collected in test tubes and centrifugated at 3000 rpm for 15 min. After then, sera were obtained and kept in the freezer at -20^{0}C until use.

Determination of total cholesterol, triglycerides, HDL in the blood of rats: Serum levels were done using a commercial kit purchased from Spin react, Ctra Santa Coloma, Spain.

Determination of low density lipoprotein (LDL-C): Serum levels of LDL-C were calculating using the formula of Friedwald *et al*.; (1972).

Statistical analysis:

Data were statistically analyzed by IBM, pc-computer using MSTAT institute program. Means were compared using Duncan's Multiple Range Test. The differences between groups were significant when P value is at $p < 0.05$ (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

In this study, *A. niger* I_4 and *A.terreus* I_8 were applied in attacking maize stalk, rice husk and wheat straw at the concentration (10 %) under solid state fermentation condition in order to obtain lovastatin (cholesterol reducing drug). Lovastatin production and optimization of fermentation parameters has been of great interest since its discovery (Sayyad and Panda 2007; Jaivel and Marimuthu 2010).

In this study**,** increasing incubation period up to 7 days at 30^{o}C on the three substrates used for both *A. niger* I_4 and *A. terreus* I_8 led to increase level of lovastatin up to a maximum value and decline in the productivity could be obtained beyond this time and temperature (Tables 1 & 2). In view with the findings of other worker, Prabhakar *et al*., (2011) obtained lovastatin yield of 730 mg/g (on wheat bran) at temperature of 30^{o}C using mutated strain of *A. terreus*. Lai *et al*. (2003) found that mutant of *A.terreus* induced lovastatin in seven days fermentation. Valera *et al*. (2005) reported that the maximum yield of lovastatin by *Asprgillus flavipes* (13.49 mg $gm^{-1)}$ dry solid was obtained after 6 days of incubation at 30^{o}C. Also, Syarifah *et al*. (2013) obtained high yield of lovastatin with *A. niger* SAR at the same temperature.

Table (1): Lovastatin Production (mg/gm) by *A .niger* I_4 and *A.terreus* I_8 allowed to grow on different agricultural wastes in relation of different incubation periods.

Substrate	Incubation period (Days)	Production of lovastatin (mg/gm)	
		Fungal isolate	
		A.niger I_4	*A.terreus* I_8
Maize stalk	4	7.45^{l}	$13.14^{h}\pm 1.5$
	7	26.36^{d}	$30.23^{a}\pm 2.2$
	10	$23.36^{e}\pm 0.38$	$25.36^{d}\pm 0.44$
	13	$18.32^{g}\pm 0.066$	$21.23^{f}\pm 0.18$
Rice husk	4	$10.68^{j}\pm 1.74$	$13.72^{h}\pm 0.26$
	7	$27.54^{c}\pm 1.0$	$28.64^{b}\pm 0.36$
	10	$23.36^{e}\pm 0.13$	$25.91^{d}\pm 0.2$
	13	$17.45^{g}\pm 0.13$	$20.45^{f}\pm 0.13$
Wheat straw	4	8.63^{k}	11.82^{h}
	7	$25.45^{d}\pm 0.04$	$30.06^{a}\pm 1.0$
	10	$23.54^{e}\pm 0.0$	$25.7^{d}\pm 0.028$
	13	$21.27^{f}\pm 0.25$	$23.82^{e}\pm 0.134$
	LSD=1.037		

Each value represents the mean of three replicates Values with unlike superscript letterers are significantly different ($p \leq 0.05$) L.S.D: Least Significant Differences**.**

ISSN: 0975-8585

Table (2): Relation of different incubation temperatures to lovastatin productivity (mg/gm) by A. niger $_{I4}$ and *A.terreus* I_8 allowed to grow on different agricultural wastes.

Fungal isolate	Incubation temperature (^{0}C)	Lovastatin yield (mg/gm)		
		Maize stalk	Rice husk	Wheat straw
A. niger I_4	10	0.01^{k}	0.01^{k}	3.4^{ij}
	20	22.01^{h}	24.1^{g}	24^{g}
	30	27.04^{efg}	27.88^{efg}	26.18^{efg}
	40	21.73^{h}	22.27^{h}	21.73^{h}
A. terreus I_8	10	4.2^{i}	4.32^{i}	4.2^{i}
	20	28.9^{a}	26.45^{de}	28.45^{ab}
	30	30.1^{a}	28.85^{c}	30.4^{a}
	40	25.186^{g}	26.18^{def}	24.63^{fg}
	LSD = 1.608			

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Table (3): Relation of different inoculum size to lovastatin Productivity by *A. niger* I_4 and *A. terreus* I_8 allowed to grow on different agricultural wastes.

Fungal isolate	Inoculum size (spores /ml)	Lovastatin yield (mg/gm)		
		Maize stalk	Rice husk	Wheat straw
A. niger I_4	$1x10^2$	20.6^{l}	16^{o}	17.18^{no}
	$1x10^4$	25.04^{ghi}	21.41^{kl}	22.74^{jk}
	$1x10^6$	25.36^{fgh}	22.53^{jk}	$25.1g^{hi}$
	$1x10^8$	27.5^{efg}	27.78^{efg}	28^{bc}
	$1x10^{10}$	18.5^{m}	24.22^{hi}	23.77^{ij}
A. terreus I_8	$1x10^2$	18.77^{m}	17.8^{no}	22.66^{jk}
	$1x10^4$	22.6^{jk}	21.36^{kl}	25.81^{fg}
	$1x10^6$	24.63^{ghi}	25.5^{fgh}	$27.32d^{e}$
	$1x10^8$	30.59^{b}	29.26^{cd}	30.32^{a}
	$1x10^{10}$	25.91^{fg}	26.46^{ef}	27.32^{de}
	LSD = 1.245			

Table (4): Relation of different pH values to lovastatin productivity (mg/gm) by *A. niger* I_4 and *A .terreus* I_8 allowed to grow on different agricultural wastes.

ungal isolate	pH	Lovastatin yield (mg/gm)		
		Maize stalk	Rice husk	Wheat straw
A. niger I_4	3	17.18	16.72	16
	4	20.45^{mn}	19.1^{o}	19.1^{o}
	5	27.91^{f}	28.54^{ef}	27.1^{g}
	6	26.14^{hi}	25.86^{i}	26.23^{k}
	7	23.54^{kl}	23.82^{kl}	23.14^{lm}
A. terreus I_8	3	23.09^{j}	20.36^{n}	21.82^{kl}
	4	26.4^{ef}	24.2^{hi}	23.77^{lg}
	5	27.05^{e}	24.43^{ghi}	24.87^{gh}
	6	29.27^{b}	28^{d}	30.72^{a}
	7	28.23^{cd}	26.62^{ef}	28.8^{bc}
	LSD = 0.7236			

Each value represents the mean of three replicates. Values with unlike superscript letterers are significantly different (p≤ 0.05) L.S.D: Least Significant Differences.

After seven days incubation both fungal isolates *A. niger* I_4 and *A. terreus* I_8 at 30^{o}C produced maximum lovastatin when inoculated with an inoculums size of 10^8 spores/ml with the three agricultural wastes under this experiment (maize stalk, rice husk and wheat straw) at pH 5 and 6 for *A. niger* I_4 and

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A. terreus I_8 respectively. Increasing inoculum size and pH more than the optimal values caused decline in production (Table 3 & 4). These obtained data are in accordance with Shindia (2001) who studied the potentiality of 25 fungal species belonging to fourteen genera from Egyptian soils to produce mevinolin and found that the maximum yield achieved by *A. terreus* with initial pH 5-6 on selected substrates. Also Wei *et al*., (2007) obtained the same result with *A. terreus* ATCC20542. Similarly Valera *et al*., (2005) obtained lovastatin by solid state fermentation using initial spore concentration of $1x10^8$ spores/ml of *Aspergillus flavipes* BICC5174. Wei *et al*., (2007) obtained maximum lovastatin yield using inoculum of spore concentration (10^7-10^8) spores/ml of *Aspergillus terreus*.

Recently the effect of carbon and nitrogen source on lovastatin production were reviewed by Bizukojc, and Ledakowicz (2005), Wei *et al*. (2007) and Jia *et al*; (2009). Concerning the influence of nitrogen source on lovastatin yield by fungal strains under this study, data obtained indicated that peptone, soyabean meal and yeast extract induced the highest production of lovastatin by *A. niger* I_4 growing on rice husk, wheat straw and maize stalk, in addition to sodium nitrate in case of maize stalk (Table 5). As for *A. terreus* I_8 also yeast extract, soyabean meal, sodium nitrate and peptone exhibited more lovastatin production with maize stalk in comparison with the control. Also soyabean meal, yeast extract and sodium nitrate in case of rice husk. Slight increase observed with soyabean meal and sodium nitrate with wheat straw for this strain (Table 5). In view with other workers Lai *et al*., (2003) found that yeast extract and sodium nitrate achieved relatively higher lovastatin production than the control (without the nitrogen addition test) by *Aspergillus terreus*.

Data obtained in this study showed that the tested carbon sources (glycerol, lactose and glucose) at concentration used (5 and 15%) gave lovastatin production more than the control for both *A. niger* I_4 and *A. terreus* I_8 with the three tested agricultural wastes (Table 6). It can be concluded that type of carbon source under the study (glycerol, lactose and glucose) affect lovastatin production for both *A. niger* I_4 and *A. terreus* I_8**.** These obtained data confirmed the work of Lo'pez *et al*., (2003) and Jia *et al*., (2009) who showed that production of lovastatin by *Aspergillus terreus* 50542 was influenced by type of carbon sources (lactose, glycerol and fructose), and the maximum quantity of lovastatin was produced (30 mg/g) when lactose was used as the carbon source. On the other hand, Sitaram Kumar *et al*. (2000), Casas Lopez *et al*. (2003) and Lai *et al*. (2003) concluded that a slowly utilized carbon source as lactose or glycerol was better assimilated for the mevinolic acid biosynthesis than glucose.

Table (5): Relation of different nitrogen sources to lovastatin Production (mg/gm) by *A.niger* I_4 and *A. terreus* I_8 allowed to grow on different agricultural wastes.

Fungal isolate	Nitrogen source	Lovastatin yield (mg/gm)		
		Maize stalk	Rice husk	Wheat straw
A. niger I_4	Control	27.91^{cde}	28.53^{de}	27.18
	NaN03	28.62^{cde}	27.55^{cde}	27.36^{cde}
	$(NH4)_2SO4$	27.18^{cde}	26.63^{de}	25.75e
	NH4Cl	26.8^{de}	26.52^{de}	26.52^{de}
	Peptone	28.81^{de}	29.95^{cde}	28.54^{de}
	Soybean meal	29.05^{de}	29.66^{d}	28.14^{de}
	Yeast extract	28.48^{cde}	29.72^{d}	28.6^{cde}
A. terreus I_8	Control	29^{e}	27.82^{e}	29.4^{d}
	NaN03	30^{d}	29.45d	29.92^{d}
	$(NH4)_2SO4$	27.41^{e}	27.77e	28.23^{e}
	NH4Cl	27.77^{e}	27.12de	29.06e
	Peptone	30.65^{c}	31ab	30.1^{e}
	Soybean meal	30.7^{c}	29.72^{d}	30^{d}
	Yeast extract	31.88^{a}	30.45^{ab}	30.33^{c}
	LSD value = 3.693			

ISSN: 0975-8585

Table (6): Relation of different carbon sources to lovastatin Productivity (mg/gm) by *A. niger* I_4 and *A.terreus* I_8 allowed to grow on different agricultural wastes.

Fungal isolate	Carbon source	Lovastatin yield (mg/gm)		
		Maize stalk	Rice husk	Wheat straw
A. niger I_4	Control	26.3	26	27.54
	Glycerol 5%	30.4	27.97	30.4
	Glycerol 15%	29.35	30.2	30.78
	Lactose 5%	29.52	30.06	30.02
	Lactose 15%	27.98	30.6	26.8
	Glucose 5%	27.9	29.7	27.57
	Glucose 15%	27.29	26.07	28.5
A. terreus I_8	Control	29	27.44	28.18
	Glycerol 5%	30.14	28.05	28.27
	Glycerol 15%	32.6	28.59	31.23
	Lactose 5%	30.6	27.74	31.74
	Lactose 15%	31.5	29.24	29.07
	Glucose 5%	29.35	27.37	28.73
	Glucose 15%	29.16	28.51	28.05

Five divalent metal cations (Fe^{+2}, Ca^{+2}, $Zn^{+2,}$ Cu^{+2} and Mg^{+2}) at two concentrations (2 and 4 mM /L) were incorporated in the production medium to study their effect on lovastatin production by *A. niger* I_4 and *A. terreus* I_8. In general it can be noticed that Fe^{+2}, Ca^{+2}, Zn^{+2} and Mg^{+2} enhanced the production for both isolates with the three substrates under experiment, while Cu^{+2} had no effect on lovastatin production for *A. niger* I_4 and reduced the production of *A. terreus* I_8 (Fig 1 & 2). Similar results obtained by Jia, *et al*., (2009*) who found that $Zn^{+2,}Fe^{+2}$ or Mg^{+2} enhanced lovastatin production markedly, while Cu^{+2} had no influence on biosynthesis of lovastatin from *Aspergillus terreus* Bizukojc *et al*. (2007) showed that supplementation of the culture media with vitamin B enhanced lovastatin biosynthesis by *Aspergillus terreus*. In this study two vitamins, B_1(thiamin) and B_6 (pyridoxine) were supplemented separately to the fermentation media of *A. nigr* I_4 and *A. terreus* I_8 at three concentration 1, 2 and 4 mg/l. Data obtained indicated that B_6 at 4mg/l induced the highest yield of lovastatin by *A. nigr* I_4, in case of maize stalk, rice husk and wheat straw in addition to B_1 and B_6 at 4mg/L for wheat straw. While, B_1 and B_6 at 4mg/l recorded the maximum yield of lovastatin for*A. terreus* I_8 on maize stalk. Also B_6 at 4mg/L recorded the best production with rice husk and B_1 at 4mg/L for wheat straw for *A. terreus* I_8 (Table 7). The influence of the supplementation of culture media with B-group vitamins on the biosynthesis of lovastatin (mevinolinc acid) by *Aspergillus terreus* ATCC 20542 suggested that B-group vitamins acted as coenzymes in the biosynthesis of lovastatin (Bizukojc *et al*., 2007). The study also showed that the significant positive effect on the biosynthesis of mevinolinic acid at concentrations not exceeding 1-5 mg/L for pyridoxine when investigated five B-group vitamins (Bizukojc *et al*., 2007).

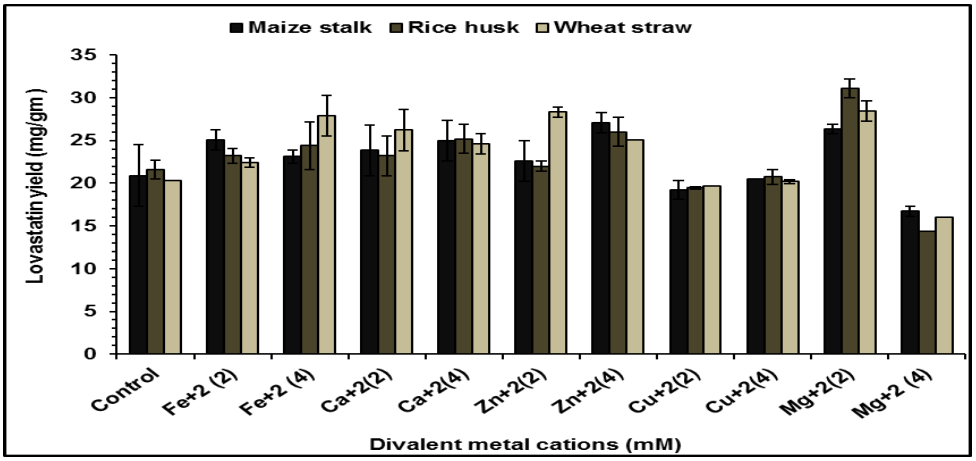

Fig. (1): Production of lovastatin (mg/gm) by *A. niger* I_4 grown on agricultural wastes in relation to different divalent metal cations.

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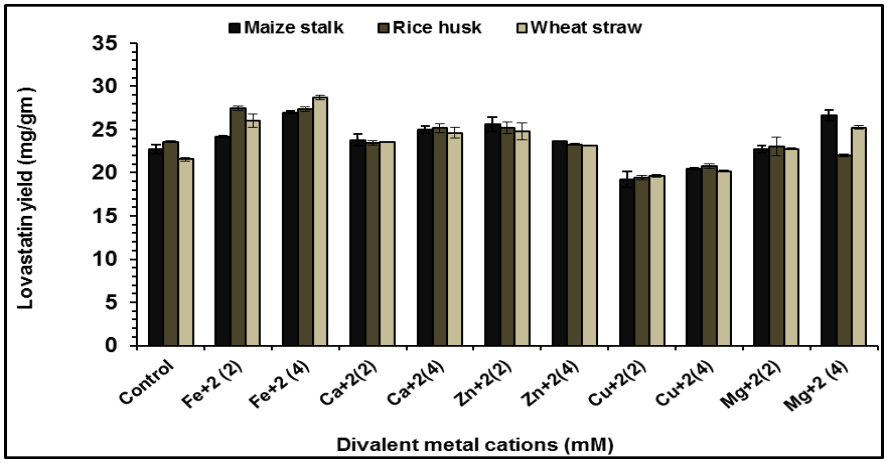

Fig.(2): Production of lovastatin (mg/gm)n by *A. terreus* I_8 grown on agricultural wastes in relation to different divalent metal cations

Table (7): Relation of vitamin B_1 &B_6 to lovastatin production (mg/gm) by *A.niger* I_4 and *A.terreus* I_8 allowed to grow on different agricultural l wastes

Fungal isolate	Vitamin	conc.(mg/L)	Lovastatin yield (mg/gm)		
			Maize stalk	Rice husk	Wheat straw
A.niger I_4	Control	0	25.9	24.79	25.3
	B_1	1	27.2	25.6	26.6
		2	28.62	27.2	27.584
		4	29.386	28.2	30.59
	B_6	1	27.8	28.4	27.388
		2	29.88	30.6	29.18
		4	31.09	31.88	30.19
A.terreus I_8	Controle	0	28.72^{def}	27.74^{fg}	29.96^{cd}
	B_1	1	25.59^{h}	27.17^{g}	27.16^{g}
		2	29.8^{bcd}	28.09^{efg}	27.20^{g}
		4	30.78^{ab}	28.60^{bcd}	30.66^{ab}
	B_6	1	28.72^{def}	27.33 ±0.27	28.05^{fg}
		2	29.643 ±0.38	27.71 ±0.085	29.6^{bcd}
		4	31.175^{a}	30.15^{abc}	29.8^{bcd}

Each value represents the mean of 3 replicates ±SE

Concurrent treatment with lovastatin inhibited the gain of body weight compered to coconut oil treated rats (hypercholesterolemic rats) (Table 8). These results suggested that the lovastatin was able to control the increase in body weight. In the presnt study, coconut oil treated rats (hypercholesterolemic rats) showed increased levels of serum cholesterol, triglycerides, LDL and increased HDL when compared with control group (Table 9). But, fungal lovastatin from *A niger* I_4 along with coconut oil was able to significantly decrease serum cholesterol, triglycerides and LDL when compared with hypercholesterolemic rats, while HDL level was increased in treated group (Table 9).

Table (8): Body weight of different groups of male albino rats

Groups	Mean weight (gm) at	
	Initial experiment	End experiment
Group-I: Normal rats	**203.5±12**	**237.6±12.4**
Group-II: Hypercholesterolemic rats	**204.4±3.1**	**241.5±8.6**
Group-III: Hypercholesterolemic lovastatin teated rats	**196.75±4.2**	**227.7±3.2** ^{ab}

(a) Significantly different from normal group, (b) significantly different from the hypercholesterolemic group. Significant at P <0.05

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Table (9): Effect of fungal lovastatin (0.4mg/day) after 8 days on serum total cholesterol, triglycerides, H.D.L and L.D.L in hypercholesterolemic rats .

Groups	Total cholesterol (mg/dl)	Triglycerides(mg/dl)	H.D.L(mg/dl)	L.D.L(mg/dl)
Group-I: Normal rats	86.7±1.69	114.4±4.54	38. 3± 1.2	25.54±2.8
Group-II: hpercholesterolemic rats	104.89±1.25[a]	165.5± 7.27[a]	34.27± 1.45[a]	37.52±4.33[a]
Group-III: Hypercholesterolemic lovastatin treated rats	84.37±2.9[b]	105.9± 8.12[b]	45.06± 3.1[ab]	18.13±5.3[b]

(a) Significantly different from normal group, (b) significantly different from the hypercholesterolemic group. Significant at $P < 0.05$

These obtained results are in agreement with **Kusmana *et al*.; (2012)** who also found that the lovastatin extract produced from *Aspergillus flavus* UICC360 decreased total cholesterol, triglycerides and LDL contents, but increased the HDL content. Generally, lovastatin is an essential compound to overcome the hypercholesterolemia disease because its activity could inhibit the hydroxymethylglutaril-coenzyme A (HMG-CoA) reductase, which serves as a catalyst in the biosynthesis of cholesterol (**Raghunath & Palaniswamy, 2015)**. HMG-CoA reductase is the main enzyme that will convert HMG-CoA to mevalonate, at the time when lovastatin in β-hydroxy-acid is at higher concentrations than HMG-CoA, thus the HMG-CoA reductase would prefer to bind with mevalonate so that the formation of mevalonate would not present, and as the consequence of that the formation of cholesterol would be inhibited **(Lai et al., 2005**).

CONCLUSION

The different cultural and nutritional parameters on lovastatin production by *A. niger*, I_4 and *A. terreus* I_8 from three agricultural wastes, maize stalk, rice husk and wheat straw under solid – state conditions were investigated. Type of nitrogen and carbon sources affected lovastatin production by both the fungal isolates. Fe+2, Ca+2, Zn+2 and Mg+2 enhanced the production for both isolates with the three substrates under experiment, while Cu+2 had no effect on the production by *A. niger* I_4 and it reduced the production by *A. terreus* I_8. Vitamins, B1 (thiamin) and B6 (pyridoxine) were supplemented separately to the fermentation media of the study and they enhanced lovastatin biosynthesis. Results obtained revealed that fungal lovastatin from *A. niger* I4 induced significant decrease in total cholesterol, triglycerides and LDL while increasing HDL level of albino rats.

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