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Screening of Some Fungal Isolates for Lipase Production and Optimization of Cultural Conditions for the Most Potent Isolate

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SIXTEEN Fungal isolates were screened for lipase production. *Trichoderma viride* was the most promising isolate for lipase activity. Lipase production has been optimized by this isolate using the following conditions; pH 6.0, temperature 25^{o}C and 6 days of incubation. Moreover, the enzyme production has been enhanced by supplementing the production medium with egg yolk as only carbon source. Starch also resulted in an increase in lipase production. The production of the lipase enzyme in medium devoid from the lipid sources proves that, this lipase is partially a constitutive enzyme. Fungal discs have been found to be the most suitable inoculum. Aeration has been proved to be favored for lipase production. The static condition was advantages than the agitation for the lipase activity. Peptone as organic N-source, sodium nitrate as inorganic N-source and KH_2Po_4 as phosphate source were more suitable for lipase production. One tenth percent oil concentration (w/v) was the best for lipase production.

Keywords: Lipase, *Trichoderma viride.*

Lipases, or triacylglycerol acyl ester hydrolases (EC 3.1.1.3), are enzymes possessing an intrinsic capacity to catalyze cleavage of carboxyl ester bonds in tri-, di- and monoacylglycerols (the major constituents of animal, plant, and microbial fats and oils). As a result of this type of reaction, carboxylic acids and alcohols with a lower number of ester bonds (and eventually glycerol) are released (Paiva *et al.,* 2000). Under natural conditions, lipases catalyze the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase where the enzyme remains dissolved. Under certain experimental conditions, such as the presence of traces of water, they are capable of reversing the reactions. The reverse reaction leads to esterification and formation of glycerides from fatty acids and glycerol (Ghosh *et al.*, 1996).

Lipases are ubiquitous enzymes which are found in most organisms from the microbial, plant and animal kingdom (Reis *et al*., 2009). Microbial lipases are commercially significant because of their advantages over lipases from other origins. Microbial lipases characterized by their stability in organic solvents, broad substrate specificity, their high enantioselectivity beside their wider

availability, relative ease of purification and lower production cost than plant and animal lipases (Pollero *et al*., 2001 and Aravindan *et al*., 2007). Fungi are potent sources of lipases. Studies on fungal lipases have been started as early as 1950 since then, many workers have exploited as a valuable source of lipase due to the following properties; thermal stability, pH stability, substrate specificity and activity in organic solvents. Fungal lipases have benefit over bacterial ones due to the fact that present day technology favors the use of batch fermentation and low cost extraction methods. In this regard, a good number of fungi have been screened for lipase production. The chief producers of commercial lipases are *Aspergillus niger, A. terreus, A. carneus, Candida cylidrocea, Rhizopus arrhizus, R. delemar, R. japonicus, R. niveus, R. oryzea, Candida rugosa, Candida antarctica , Thermomyces lanuginosus* and *Rhizomucor miehei* (Ghosh *et al*., 1996 ; Adnan , 1998 and Sharma *et al*., 2001).

The participation of lipases in the worldwide enzyme industry market has grown significantly and it is believed that, in the future, they will acquire importance comparable to that of the peptidases, which currently represent 25 to 40% of industrial enzyme sales (Barros *et al.*, 2010) that due to their multifaceted properties, which find usage in a wide array of industrial applications and biomedical sciences (Gupta *et al.*, 2004). Thus, that demand for lipases with different characters stimulates the isolation and the selection of strains of high lipolytic activities and optimization of its production.

Materials and Methods

Microorganisms

The fungal strains were locally isolated from air in the area of Helwan University, soil samples collected from different places (Saloja – Tepene - El-Alag -Helwan University 'round oily plant') and others were obtained as pure cultures from Botany and Microbiology Department – Faculty of Science – Helwan University.

The isolated fungi were identified at least in terms of genus level depending on their morphological characters on different culture media (Czapeks-Dox agar medium, potato dextrose agar medium and malt extract agar medium) and microscopic examination for conidia and hyphae according to Barnett & Barry (1987) and Pitt (1979). One isolate was identified in Regional Center for Mycology and Biotechnology, Alazhar University as *Trichoderma viride.*

Fungal screening for lipase production

This test was carried out using Rhodamine B-olive oil medium described by Kouker & Jaeger (1987) which contains (g/l): Nutrient broth 8, NaCl 4, Agar 10, pH 7, olive oil 31.25 g, Rhodamine B solution (0.01 % w/v) 10 ml as follows:

Twenty ml of Rhodamine B–olive oil medium was poured into sterile petri dishes then inoculated with 0.8 cm fungal discs from 48 hr old culture, then the plates were incubated at 28°C for 48 hr. Lipase production was detected by

exposing the plates to UV light at 350 nm. The fungal colonies that have lipolytic activity show orange fluorescent halo as a result of the interaction of Rhodamine B with the fatty acids released during the hydrolysis of olive oil by lipase enzyme.

Selection of the potent isolate for lipase production

Medium contained (g/l): $MgSo_4.7H_2o$ 0.5, KH_2Po_4 1, $NaNo_3$ 3, Peptone 30, $CaCl_2.2H_2o$ 0.5, Olive oil 0.2% v/v, pH= 5 was used for selection of the potent isolate for lipase production as follows:

Conical flasks (250 ml capacity) containing 50 ml of the above medium were autoclaved. Each flask was inoculated with 2 fungal discs (0.8 cm) of 4-days old culture and incubated at 28°C for 5 days. The cultures were filtered using filter paper Whatman no.1 for the removal of fungal growth. The filtrate was used as the source of the crude enzyme.

Asssay

Lipase activity was determined by modified paranitrophenyl- palmitate (pNPP) method as described by Licia *et al.* (2006) as follows:

Reaction mixture of one ml of the pNPP emulsion was added to 1.9 ml of phosphate buffer (0.1 M, pH 7) and 0.1 ml of the crude enzyme solution (filtrate), then incubated at 30°C for 1hr, then the reaction was stopped by adding 0.2 ml of isopropanol. The developed color has been measured at 410 nm against blank (enzyme free reaction mixture). One unit of lipase is defined as the amount of the enzyme which librates 1 µmole pNPP per minute under the assay conditions. pNPP standard curve was prepared in the range of 10-100 µmole.

Dry biomass measurement

Fungal biomass determination was carried out by filtration through preweighted filter paper Whatman no. 1 then washed with distilled water then dried in oven until obtaining 2 successive equal weights for the same sample. The biomass was calculated in terms of gram per liter of growth medium.

Effect of lipid sources

Different lipid sources were tested by supplementing the production medium with 0.2 %(w/v) of oil and lipid sources, *i.e.* raw corn oil, corn oil (commercial), sweet almond oil, peanut oil, sesame oil, different commercial olive oils, camphor oil, mill of corn germ and egg yolk. At the end of the incubation period the fungal biomass was separated from the growth medium by filtration and the filtrate was used as a crude enzyme solution for the assessment of the lipase activity.

Effect of incubation periods

Conical flasks (with 250 ml capacity) occupied with 50 ml of the standard production medium were autoclaved and inoculated with two 0.8 cm fungal discs then incubated at 28°C for 3, 4, 5, 6 and 7 days.

Effect of inoculum type and size

Conical flasks (with 250 ml capacity) occupied with 50 ml of the standard production medium were autoclaved and inoculated with different inoculum types (fungal discs and fungal spore suspention (3×10^4)), where each inoculum type was tested with different sizes (1,2,4,6 (0.8 cm) discs – 1,2,4 ml spore suspension (3×10^4)). At the end of the incubation period the fungal biomass and the enzyme activity were determined.

Effect of incubation temperature

Flasks with 50 ml of the sterilized standard production medium were inoculated and incubated at different temperatures, *i.e.* 15, 20, 25, 28, 30, 35, 40°C. At the end of the incubation period the fungal biomass production and the enzyme activity were determined.

Effect of initial pH values

Initial pH value of the medium has been adjusted with diluted acid (1N HCl) and base (1N NaoH) in the range of (pH 4, 5, 6, 8 and10). At the end of the incubation period the fungal biomass production and the enzyme activity were determined.

Effect of carbon sources

The oil was replaced with different carbon sources, *i.e.* glucose - fructose - sucrose – manitol – maltose – starch - cellulose at a concentration of 1% (w/v) and added in equimolecular weights to test their effect as a sole C-source of the medium.

Also, the used C-sources were added to the medium in the presence of oil.

The control in both cases was the medium with oil as the sole C-source. At the end of the incubation period the fungal biomass production and the enzyme activity were determined.

Effect of nitrogen sources

Effect of inorganic N-sources, *i.e.* sodium nitrate, sodium nitrite, ammonium nitrate, ammonium sulfate, ammonium chloride, diammonium hydrogen orthophosphate and potassium nitrate (which added with the same concentration in equimolecular weights), As well as, effect of organic N- sources, *i.e.* peptone corn steep liquor and yeast extract (that added with the same concentration) was investigated. At the end of the incubation period the fungal biomass production and the enzyme activity were determined.

Effect of oil concentration

The medium was supplemented with different concentrations of oil, *i.e.* 0.05, 0.1, 0.4 and 0.6 % w/v. At the end of the incubation period the fungal biomass production and the enzyme activity were determined.

Effect of aeration

Different volumes of the production medium, *i.e.* 20, 40, 50, 60, 80 and 100 ml were distributed into 250 ml conical flaks. At the end of the incubation period the fungal biomass production and the enzyme activity were determined.

Effect of phosphate sources

The phosphate source in the medium (KH_2Po_4) was omitted from the medium and replaced by other phosphate sources with the same concentration in equimolecular weight such as (K_2HPo_4, NaH_2Po_4, Na_2HPo_4, $NH_4H_2Po_4$ and $(NH_4)_2HPo_4$). The control flasks were those of the normal phosphate source (KH_2Po_4).

Effect of agitation

The flasks contained 50 ml of the sterilized production medium were inoculated as usual but one group was incubated under static conditions, while the other group was incubated under shaking conditions (80 rpm). At the end of the incubation period the fungal biomass production and the enzyme activity were determined.

Statistical analysis

Statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by homogenous subsets (Duncun[a]) at confidence level of 5% (0.05) using the Statistical Package for the Social Science (SPSS) version8. Duncan's multiple range tests were used to compare between means of treatments according to Walter & Duncan (1969) at probability 5%.

Results and Discussion

Microorganisms

The fungal strains were locally isolated from air in the area of Helwan University, *i.e. Cladosporium* sp. (three isolates), *Aspergillus* sp. (two isolates), *Helminthosporium* sp. (two isolates) and *Aspergillus flavous*, from soil samples collected from different places such as from Saloja: *Paecillomyces* sp. and *Aspergillus niger* 1, from Tepene: *Trichoderma viride* and *Penicillium* sp., from El-Alag: *Aspergillus ochraceous* and finally from Helwan University 'round oily plant': *Aspergillus niger* 2 and others were obtained as pure cultures from Botany and Microbiology Department – Faculty of Science – Helwan University, *i.e. Fusarium* sp. and *Cylendrocarbon radicicola*.

Fungal screening for lipase production

The colonies of true lipase production formed orange fluorescent halo when exposed to U.V. light (350 nm) as shown in Fig. 1. Results were recorded in Table 1. *Paecillomyces* sp., *Aspergillus niger* (1), *Aspergillus flavous, Fusarium* sp., *Trichoderma viride, Penicillium* sp. and *Aspergillus niger* (2) showed positive lipase production.

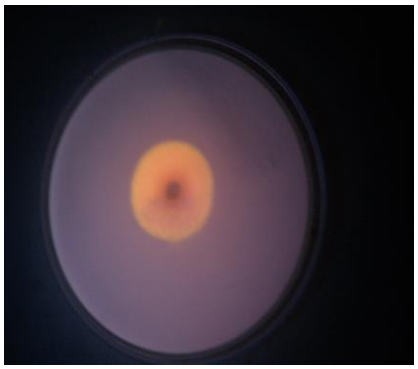

Fig. (1-a). *Aspergillus flavous* showed fluorescent orange halo under U.V, 350 nm, on Rhodamine B- oil medium (+ ve lipase activity).

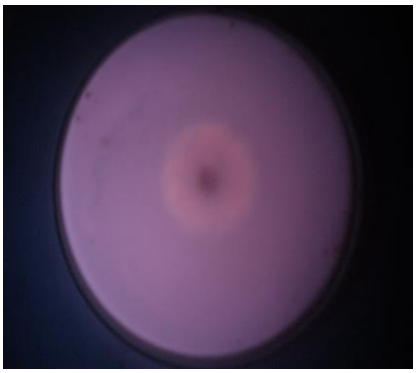

Fig. (1-b). *Cylendrocarbon radicicola* showed no fluorescent orange halo under U.V, 350 nm, on Rhodamine B-oil medium (- ve lipase activity).

TABLE 1. Lipase production using Rhodamine B-oil medium.

Isolate	Result
Cladosporium sp. (1)	- ve
Aspergillus sp.(1)	- ve
Helminthosporium sp. (1)	- ve
Helminthosporium sp. (2)	- ve
Aspergillus sp. (2)	- ve
Cladosporium sp. (2)	- ve
Cladosporium sp. (3)	- ve
Paecillomyces sp.	+ ve
Aspergillus niger (1)	+ ve
Aspergillus flavous	+ ve
Fusarium sp.	+ ve
Cylendrocarbon radicicola	- ve
Trichoderma viride	+ ve
Penicillium sp.	+ ve
Aspergillus niger (2)	+ ve
Aspergillus ochraceous	- ve

Selection of the potent isolate for lipase production

The results of Table 2 showed that, the most potent isolate for the lipase production was *Trichoderma viride.* Thus this isolate was selected for the further study.

TABLE 2. Lipolytic activities of the lipase producing fungi.

Isolate	Lipase units/ml	Dry weight g/l
Aspergillus flavous	6.29	1.30
Aspergillus niger (1)	5.73	1.76
Aspergillus niger (2)	3.15	1.64
Trichoderma viride	10.10	1.70
Fusarium sp.	3.74	1.82
Paecillomyces sp.	2.36	1.80
Penicillium sp.	0.08	0.64

Effect of lipid sources

The presence of lipid sources in the production medium has been proved to enhance the lipase production (Hasan *et al.*, 2009). The results represented in Table 3 showed that the maximum lipase production has been maintained when the egg yolk used as lipid source (19.88 u/ml activity) followed by peanut oil which gave 11.7 u/ml activity. The addition of egg yolk also has been proved to enhance lipase and biomass production by *Rhizopus arrhizus* (Adnan, 1998). This may be due to the high nutrient ratio which includes cholesterol and triglycerides (Morrison, 1951). Simultaneously the presence of these two substances in the fermentation medium have stimulatory effect on fungal growth and lipase production as reported by Valero *et al.*(1988).

TABLE 3. Effect of lipid sources on biomass and lipase production by *T.viride* .

Oil/ lipid source	Lipase units/ml	Dry weight g/l
Raw corn oil	8.15^{cd}	2.38
Corn oil	6.23^{efg}	1.50
Sweet almond oil	5.00^{g}	2.42
Peanut oil	11.70^{b}	2.36
Sesame oil	5.26^{g}	2.84
Olive oil 1	8.62^{c}	1.72
Olive oil 2	7.35^{cde}	1.98
Olive oil 3	7.25^{cde}	2.22
Camphor oil	6.80^{def}	2.36
Egg yolk	19.88^{a}	8.90
Mill of corn germ	2.83^{h}	4.00

Effect of incubation periods

Trichoderma viride showed the maximum lipase and biomass production in the 6^{th} day of incubation as shown in Table 4, then lipase production decreased significantly. This in agreement with the hypotheses that, the highest lipase activity usually related to the period of maximum vegetative growth (Ahmed, 2008) and a decrease in the enzyme activity occurred simultaneously with the onset of the stationary phase of the fungal growth (Nahas, 1988). That maximum

lipase production in the 6th day also has been observed in case of the Endophytic fungus *Cercospora kikuchii* (Silva *et al*., 2011). In contrast, *Aspergillus japonicus* showed highest enzyme activity and biomass production in the 7*th* day (Jayaprakash *et al*., 2010).

TABLE 4. Effect of incubation periods on biomass and lipase production by *T. viride*.

Incubation period (Day)	Lipase units/ml	Dry weight g/l
3*rd*	4.58[e]	0.82
4*th*	7.33[d]	2.50
5*th*	11.38[b]	2.50
6*th*	15.17[a]	3.30
7*th*	9.15[c]	3.30

Effect of inoculum type and size

Maximum lipase activity has been obtained by the addition of 4 fungal discs as well as 2% spore suspension (3×10^4), other inoculum sizes showed variable results (Table 5). Variable observations have been reported for other fungal species. Adnan (1998) reported that 4% spore suspension (4×10^4) was the most effective in case of *Rhizopus arrhizus* while, *Penicillium simplicissimum* showed maximum lipase production by addition of 10 ml spore suspension (10^7) (Gutarra *et al.*, 2007).

TABLE 5. Effect of inoculum type and size on biomass and lipase production by *T. viride.*

Inoculum	Lipase units/ml	Dry weight g/l
1 disc	10.15[c]	2.28
2 discs	11.60[abc]	2.06
4 discs	12.18[a]	3.80
6 discs	10.48[bc]	3.06
2% spore suspension	12.77[a]	1.94
4% spore suspension	11.72[ab]	1.06
8% spore suspension	11.40[abc]	1.34

Effect of incubation temperature

Temperature is a one of the most important physical/environmental factors influence the enzyme production. It has been observed that, the optimum incubation temperatures for lipase production by *Trichoderma viride* was 25 and 28°C but the maximum biomass production has been obtained at 20°C as shown in Table 6. In agreement with the present results, *Rhizopus oligosporus*, *Corynebacterium paurometabolum* MTCC 6841 and *Nomuraea rileyi* showed the maximum lipase production at 25°C (Nahas, 1988, Joshi *et al*., 2006 and Supakdamrongkul *et al.*, 2010). In contrast, optimum incubation temperature for lipase production by *Phoma glomerata* was 40°C (Pollero *et al.*, 2001) and that for *Tlaromyces emersonii* was 45°C (Ghosh *et al.*, 1996).

TABLE 6. Effect of incubation temperature on biomass and lipase production by *T. viride*.

Temperature (°C)	Lipase units/ml	Dry weight g/l
15	0.14[d]	0.10
20	4.25[c]	4.30
25	11.90[a]	2.90
28	11.85[a]	3.30
30	5.78[b]	2.70
35	3.90[c]	2.50
40	0.45[d]	No growth

Effect of initial pH

Initial pH of the cultural medium is crucial for enzyme production. Most fungi show optimum growth and maximum lipase production at slightly acidic pH (Jayaprakash *et al.*, 2010 and Adnan, 1998).The results obtained in this study showed that, *Trichoderma viride* exhibited maximum lipase production at pH 6.0, (Table 7) which is in agreement with the results obtained for *Calvatia gigantean*, *Aspergillus wentii*, *Rhizopus nigricans* (Ghosh *et al.*, 1996), *Fusarium oxysporium* and *Aspergillus oryzae* (Saad *et al.*, 2005). In contrast, some yeasts show maximum lipase production at pH 3.0 (Adnan, 1998). On the other extreme, *Penicillium cadidum* and *Saccharomyces lipolytica* showed maximum lipase production when the initial pH value of the medium was adjusted to pH 9.0 and 9.5, respectively (Hasan *et al.*, 2009 and Jonson & Snygg, 1974).

TABLE 7. Effect of different initial pH values on biomass and lipase production by *T. viride*.

Initial pH	Final pH	Lipase units/ml	Dry weight g/l
4.00	8.00	12.23[ab]	2.40
5.00	8.10	11.68[b]	2.26
6.00	8.60	13.95[a]	2.50
8.00	8.30	3.90[c]	Sparse growth
10.00	9.30	3.38[c]	Sparse growth

Effect of different carbon sources

The main C-source of the medium (oil) was replaced with other C-sources to test their effect as a sole C-source on enzyme production. It has been observed that, starch was the best C-source for lipase production followed by the oil as a sole C-source as shown in Table 8. On the other hand, addition of these C-sources to oil containing medium has been resulted in a depression in lipase production in case of glucose, fructose, sucrose, manitol and cellulose, while lipase production has been enhanced when the medium was supplemented with

starch and maltose (Table 8). In general the best lipase production was obtained when the medium was supplemented with both oil and starch as C-sources. The present results are similar to that obtained by Iwai *et al.* (1975) and Espinosa *et al.* (1990) who reported that, starch and dextrin were most potent carbohydrates for maximum lipase formation. In contrast, Thota *et al*. (2012) reported that, the presence of starch leads to a decrease in lipase production by *Rhizopus* sp. of more than 35-50%.

TABLE 8. Effect of different carbon sources on biomass and lipase production in presence of C-sources alone as well as in presence of oil by *T. viride*.

C-source	Lipase units/ml	Dry weight g/l
Oil	11.15^{cd}	2.38
Glucose	6.80^{fg}	5.82
Fructose	8.05^{e}	4.56
Sucrose	10.50^{d}	5.64
Maltose	10.12^{d}	1.84
Manitol	6.10^{g}	5.64
Starch	11.88^{bc}	2.20
Cellulose	8.61^{e}	2.80
Oil + glucose	7.78^{ef}	8.00
Oil + fructose	8.35^{e}	6.90
Oil + sucrose	7.95^{e}	7.84
Oil + maltose	12.68^{b}	3.58
Oil + manitol	7.56^{ef}	7.00
Oil + starch	14.30^{a}	5.60
Oil + cellulose	10.18^{d}	3.40

Effect of nitrogen sources

In a trail to investigate the effect of N-sources on lipase production, peptone and corn steep liquor have been found to be the best organic N-sources for lipase production by *Trichoderma viride*, but yeast extract decreased the lipase production (Table 9). The results are in agreement with that obtained for *Aspergillus fumigatus, A. terreus, Penicillium chrysogenum, P. funiculosum and Fusarium moniliforme*, where peptone was the best N-source (Rani & Panneerselvam, 2009). For the inorganic N-source, the maximum lipase production has been maintained in presence of sodium nitrate, followed by potassium nitrate (Table 9). The results are similar to that obtained for lipase production by *Antroda cinnamomea* (Lin *et al.*, 2005).

TABLE 9. Effect of inorganic and organic N-sources on biomass and lipase production by *T. viride*.

N-source	Lipase units/ml	Dry weight g/l
Sodium nitrate	11.40[a]	2.64
Sodium nitrite	7.8[bc]	2.64
Ammonium nitrate	8.70[bc]	2.96
Ammonium sulfate	8.18[bc]	2.20
Ammonium chloride	8.6[bc]	2.20
Diammonium H.Ph.	8.60[bc]	3.40
Potassium nitrate	10.73[a]	3.80
Without inorganic N-source	8.93[b]	2.80
Peptone	11.40[a]	2.64
Corn steep liquor	11.05[a]	1.92
Yeast extract	6.88[cde]	4.60
Without peptone	6.23[de]	0.034

Effect of oil concentration

The results showed that, *Trichoderma viride* characterized by its ability to produce lipase enzyme even in absence of oil or other lipid sources in the medium which proves that the lipase enzyme of *Trichoderma viride* is produced constitutively. This also has been observed for many fungal species such as many *Aspergillus* and *Rhizopus* species (Dalmau *et al.*, 2000). In contrast, other fungal species produce lipase enzyme only in presence of oil as inducer for enzyme production such as *Penicillium aurantiogriseum* which showed no lipase production in absence of oil (Lima *et al.*, 2003). However, the addition of oil to the fermentation medium has been found to enhance the lipase production in the present study which means that lipase has been produced by *T. viride* constitutively as well as inductively.

Increasing oil concentration in the medium has led to increase in lipase production up to 0.1% v/v concentration, beyond this concentration the lipolytic activity has decreased but the biomass production still increasing (Table 10). This response is in agreement with previous studies suggesting that an excess of lipid substrate causes a negative effect on lipase synthesis (Colin *et al.*, 2010). Also, *Penicillium expansum* yields maximum lipase activity when grown in medium containing 0.1% olive oil (Sztajer *et al.*, 1993). While, *Aspergillus niger* MYA 135, showed maximum growth at olive oil concentration of 3.5%. On the other hand, the highest specific activity of lipase was detected using 2% olive oil, after which it showed a decline (Gulati *et al.*, 1999).

TABLE 10. Effect of oil concentration on biomass and lipase production by *T. viride.*

Oil concentration (% v/v)	Lipase units/ml	Dry weight g/l
0.05	10.40^{ab}	2.22
0.10	11.43^{a}	2.26
0.20	11.18^{ab}	2.76
0.40	10.43^{ab}	4.40
0.60	10.07^{b}	5.60
Without oil	7.18^{c}	1.76

Effect of aeration

Aeration is a decisive factor in aerobic fermentation, thus lipase production was affected by the changes in volume ratio and shaking conditions. Lipase production by *Trichoderma viride* was maximum at the low volumes (20-60 ml fermentation medium found in 250 ml flasks), and the enzyme activity decreased with the decrease in air volume. On other hand, the biomass production increases with increasing fermentation medium volume (Table 11). These observations are similar to that reported for maximum lipase production by *Fusarium oxysporium* which was 20 ml fermentation medium/250 ml flasks and also the activity gradually decreased by increasing volume (Rifaat *et al.*, 2010).

TABLE 11. Effect of aeration on biomass and lipase production by *T. viride*.

Volume (ml)	Lipase units/ml	Dry weight g/l
20	13.40^{a}	1.50
40	11.50^{a}	3.00
50	11.90^{a}	4.60
60	11.93^{a}	4.80
80	6.40^{b}	7.40
100	4.40^{b}	12.60

Effect of phosphate sources

Several phosphate sources were compared for lipase production by *Trichoderma viride.* It has been observed that, the phosphate source of the standard fermentation medium (KH_2Po_4) was the best phosphate source for lipase production followed by $(NH_4)_2HPo4$, K_2HPo_4 and $NH_4H_2Po_4$ while the Na_2HPo_4 reduced the lipase production. In contrast, the highest lipase activity was obtained when Na_2HPo_4 was used as phosphate source in case of *Botryosphaeria ribis EC-01* (Barbosa *et al*., 2011).The highest biomass production was obtained when KH_2Po_4 was used as phosphate source (Table 12). The results are in agreement with that of *Botryosphaeria ribis EC-01* biomass production was highest on KH_2Po_4 (Barbosa *et al*., 2011).

TABLE 12. Effect of phosphate sources on biomass and lipase production by *T.viride*.

Phosphate source	Lipase units/ml	Dry weight g/l
KH_2Po_4	11.33^{a}	2.58
K_2HPo_4	9.35^{abc}	2.46
NaH_2Po_4	7.48^{bc}	1.58
Na_2HPo_4	6.23^{c}	2.64
$NH_4H_2Po_4$	9.45^{abc}	1.74
$(NH_4)_2HPo_4$	10.10^{ab}	1.96

Effect of agitation

Concerning the shaking/agitation effect, *Trichoderma viride* showed the best growth and lipase production under static conditions and dramatically decreased under shaking conditions (Table 13). These results are similar to that for lipase production by *Talaromyces emersonii* and *Mucor racemonsus* which favored the stationary conditions for maximum lipase production (Ghosh *et al.*, 1996). In contrast, shaking enhances the production of intra and extracellular lipase from thermophilic *Rhizopus oryzae* (Salleh *et al.*, 1993) also *Aspergillus wentii* and *Mucor hiema*lis showed maximum lipase production when cultured under shaking conditions (Ghosh *et al.*, 1996).

TABLE 13. Effect of agitation on biomass and lipase production by *T. viride*.

Incubation condition	Lipase units/ml	Dry weight g/l
Static	11.30	2.58
Shaking (80 rpm)	7.85	1.38

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مسح بعض العزلات الفطرية لإنتاج إنزيم الليبيز وتحسين الظروف الإنتاجية لأفضل العزلات المنتجه له

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تناول البحث عزل ست عشرعينة فطرية من الهواء ومن عينات تربة مختلفة ثم تم إختبار قدرتهم على إنتاج إنزيم الليبيز و الإستيريز. و قد أظهرت عزلة *التريكودرما فيريدى* إنتاجية عالية لإنزيم الليبيز. و قد أختيرت تلك العزلة الفطرية لإجراء المزيد من البحث حيث تم دراسة بعض العوامل البيئية والكيميائية للحصول على أعلى إنتاجية من هذا الإنزيم . فقد أدى تحضين الفطر عند درجة حرارة ٢٥ و ٢٨ درجة مئوية وعند درجة حامضية ٦,٠ ولمدة ستة أيام لتحسين إنتاجية تلك الإنزيم. وعندما تم إضافة مح البيض كمصدر دهنى للوسط الغذائى للفطر تم رفع إنتاجية الإنزيم ليصل ٢٠ وحدة لكل ملليلترمن الوسط الغذائى. بينما إضافة النشا للوسط الغذائى قد أدى إلى تحسين إنتاجية الإنزيم من ١١,١٥ وحدة لكل ملليلتر إلى ١٤,٣٠ وحدة لكل ملليلتر. وقد أظهرت الدراسة أن تلك الإنزيم هو إنزيم قوامى وليس إستحثاثى حيث تم ملاحظة قدرة تلك العزلة الفطرية على إفرازه حتى فى غياب وسطه الأساسى (الدهون). و بأختبار أحسن حقنة فطرية يمكن إستخدامها لتحسين إنتاجية الإنزيم فقد وجد أن الحقنة الفطرية المكونة من ٤ أقراص فطرية (قطرها=٠,٨ سنتيميتر) أو الحقنة الفطرية المكونة من ١ ملليلتر من المعلق الجرثومى ($٣x١٠^{٤}$) قد أدت إلى تحسن الإنتاجية. وقد وجد ان استخدام الاحجام المنخفضة من الوسط الغذائى والتحضين تحت ظروف ساكنة هى أفضل ظروف لتحسن إنتاجية هذا الإنزيم. وبإختبار المكونات الكيميائية للوسط الغذائى فقد وجد أن الببتون ونترات الصوديوم هى مصادرالنيتروجين المفضلة لإنتاج هذا الإنزيم وأن KH_2Po_4 هو مصدر الفوسفات المفضل أيضا وهذه المكونات الكيميائية تتطابق مع المكونات المثلى الموصى بها فى الوسط الغذائى المصمم لإنتاج هذا الإنزيم. بينما عند تغيير نسبة الزيت فى الوسط الغذائى إلى ٠,١ ملليليتر فإن إنتاجية الفطر لهذا الإنزيم قد اظهرت ارتفاعا.