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Biological Control of Pink Bollworm *Pectinophora gossypiella* (Saunders) by protease and Lipase Enzymes Produced from *Streptomyces vinaceusdrappus* SA (AB857336)

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STREPTOMYCES vinaceusdrappus SA was subjected to submerged fermentation under optimum conditions, at 30°C and pH 7.0 in presence of 1% casein & 0.5% maltose and 0.5 % yeast & 1% sucrose for 6 and 5 days for protease and lipase production, respectively, under static conditions. Partial purification of protease and lipase were carried out by70% ammonium sulphate precipitation, dialysis, Sephadex G_{100} and finally Sephadex G_{50}. The specific activities of protease and lipase at final purification step were 116.7 and 104.9 U mg^{-1} protein compared to 8.4 and 8.9 U mg^{-1} crude proteins, with yield 37 and 29 %, respectively. Both enzymes gave single homogenous band of 71 kDa by SDS- PAGE. Also, their optimum pH was 7.0 and they had pH stability from 7.0 – 11.0. The maximum enzyme activities obtained at 55°**C** and 60°C with Tm (half life temperature) values 86.5 and 83.9°C for protease and lipase, respectively. The penetration of newly hatched larvae of pink bollworm into the cotton bolls after being dipped in crude or purified protease and lipase each alone or in mixture (v/v) were studied. The results revealed that the penetration percent decreased in pure enzymes than crude. Treatment with the mixed crude enzymes decreased the percentage of penetration than of each enzyme alone. Also, crude lipase **,** protease and their mixture had repellent action for pink bollworm moths. Moreover, there was highly significant effect on the deformed adult percentage compared with control since the pure enzyme had effective role than crude enzyme.

Keywords: Biocontrol, *Pectinophora gossypiella*, Larval and Pupal mortality percent, Protease and Lipase enzymes, Kinetic properties.

Pesticides in modern agriculture are under pressure to be removed from the market because of their hazardous impact on the natural environment. So, biological control agents, either by effective microorganisms or microbial products, have long been attracting attention as alternatives to chemical agents (Mahfouz and Abou El-Ela 2011). Well-know bacterial agents which have been used successfully for insect control are *Bacillus thuringeinsis*, *Bacillus sphaerisus* and *Bacillus subitis* (Ghribi *et al*., 2012).

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Enzymes are gradually replacing the use of harsh chemicals in various industrial processes **(**Malathu *et al.*, 2008). Proteases which hydrolyze peptide bonds of proteins are also known as peptidyl- peptide hydrolase constitute 60-65% of the global enzyme (Genkel and Tarib, 2006). Also Proteases are obtained from plants, animal organs and microorganisms (Sevinc and Demirkan, 2011). Proteases produced from *Streptomycetes* are the most important group of secondary metabolites that are widely exploited (Limkhada *et al*., 2010). Actinomycetes are Gram-positive, mycelium-forming soil bacteria that include many species considered to be among the most important producers of protease (Balachandran *et al*., 2012).

Lipases, particularly microbial ones, are mostly extracellular and their production is greatly influenced by the medium composition, they have the ability to hydrolyze ester bonds (Nigam and Pandey, 2009). They hydrolyze substrate not only in an emulsified form but also in a micelle state (Reetz, 2002). Lipase plays a key role in the biological turnover of lipid and functioning of biological membranes and the most lipase-producing microorganisms are *Bacillus*, *Pseudomonas*, *Staphylococcus* and S*treptomyces* (Treichel *et al*., 2010).

Insect cuticle is the infection site for microorganisms applied for biological control on insects. Lipases, chitinases, proteases as well as other lysozyme enzymes are commonly produced and secreted by microorganisms to degrade the insect cuticle (Pedrini *et al*., 2006).

Recently, screening, production and formulation of entomo-pathogenic microorganisms with the potentiality for production of insect cuticle degrading enzymes have been received much attention, due to their low deleterious actions on plants, animals and humanbeings. It appears also that microbial degradation of insect protein and lipid (the main component of the insect cuticle) as well as production of proteases and lipases have captured the world wide attention of biocontrol of insect and become the objective of extensive research **(**Sahebani and Hadavi, 2008). El-Sayed (2008) studied the biological control of pink bollworm *Pectinophra gossypiella* using protease enzyme produced from microorganisms, also Lokma (2010) detected the lipases as biocontrol agent against corn leaf aphid *Rhopaosiphum maidis*.

The aim of this study was to produce, purify and characterize proteases and lipases of *S. vinaceusdrappus.* Studying the latent effect of several biological aspects and capabilities of the purified enzymes each separately or in mixture to degrade the cuticle of *Pectinophra gossypiella* insect. Also to enable the use of purified enzymes as biological control of the insects.

Material and Methods

Tested organism

Streptomyces vinaceusdrappus SA (AB857336) isolated from soil and mostly active against *Pectinophra gossypiella* (Reda *et al*., 2013) was used through this investigation.

Optimization of culture conditions for protease and lipase production

Streptomyces vinaceusdrappus SA was cultivated for protease production on starch nitrate broth medium with replacing of $NaNO_3$ by 0.2% gelatin (Ammar *et al*., 1991) . For lipase production the tested organism was subcultured on Dox-yeast extract-tributyrin broth medium (Elwan *et al*., 1977). The production of each enzyme was tested at different incubation periods (3-8 days), different temperatures (25-55°C), different pH-values (pH 4 -12) under shaking and static conditions and in presence of different carbon and nitrogen sources (Kaur *et al*., 2001).

Assay of enzyme activity

Protease enzyme activity (U/ml) of the cell-free filtrate was determined using the method of Thangam and Rajkumar (2000). This method is a modified method of Anson (1938). While, lipase activity was determined by titrimetric method as described by Nahas (1988) and modified by Yu *et al.* (2007).

Protein determination

Protein content was estimated by the method of Lowery *et al.* (1951) using bovine serum albumin (Sigma chemical Co.) for standard curve.

Protease and lipase purification

Unless otherwise stated, all procedures of protease and lipase purification were carried out at 4°C. The culture supernatant was first mixed with ammonium sulphate 70 % concentration, with constant and continually stirring (Phibbs and Bernlohr, 1971). The precipitate was then separated by centrifugation at 6000 rpm for 20 min, and the protein was then re-suspended in small amount of sodium phosphate buffer (pH 8.0). As mentioned by Ammar (1975) the re-suspended pellets were applied to a Sephadex G_{100} column then Sephadex G_{50} column which pre-equilibrated with the same buffer.

SDS-PAGE Analysis

The molecular weights of protease and lipase enzymes from culture of *S. vinaceusdrappus* SA were carried out using SDS-PAGE according to Laemmeli (1970). PageRuler Unstained Protein Ladder, Fermentas marker was used.

Biochemical properties of S. vinaceusdrappus SA purified protease and lipase

The optimum temperatures and thermal stability of the tested enzymes were determined after incubation the reaction mixture at different temperatures (30-80°C). The enzymes activities were determined for each temperature as described previously.

The thermal inactivation rate (Kr) can be described by the first-order kinetic model (Whitaker, 1972); Ln (A_t/A_o) k_tT, where A_o and A_t are the specific activity at zero and t time. The temperature at which the enzyme loss 50% of its activity (Tm) was calculated from the linear equation of different preincubation temperature at 60 min.

The optimum pH and pH stability of the tested enzymes was determined after incubation the reaction mixture at different pH values (4-12).

The biocontrol of Pectinophra gossypiella using S. vinaceusdrappus SA crude and purified protease and lipase

In these experiments the treatments was carried out by crude and purified protease with specific activities 8.4 and 116.7 U mg^{-1} protein, respectively and the used crude and purified lipase had specific activities 8.9 and 104.9 U mg^{-1} protein, respectively.

Effect of crude and purified enzymes on penetration of newly hatched larvae of pink bollworm in cotton bolls

This experiment aimed to evaluate successful penetration of newly hatched larvae of pink bollworm into cotton bolls after dipping bolls in crude and pure protease and lipase separately or in mixture (v/v). Cotton bolls age (2-3 week old) were collected from a cotton field in Sharkia Governorate during the first week of July 2013 and brought to the laboratory. Bolls were dipped in protease and lipase for 20 seconds, while control bolls were dipped in water only. Enzyme from treated and untreated bolls were left to dry in air under laboratory condition. One newly hatched larva was placed in clean glass jar with a single cotton boll and covered by fine paper and kept under laboratory condition. Ten replicates were prepared for each enzyme treatment as well as control. After 48 hr from treatment the number of pink bollworm larvae successfully penetrated and lived within green cotton bolls in each treatment and control were counted (Elmedany, 2013).

a- Repellent effect of crude and purified enzymes on pink bollworm moths

This experiment aimed to evaluate the repellent effect of the enzymes on pink bollworm moths. Cotton bolls (2-3 week-old) were collected from the cotton field in Sharkia Governorate during the first week of July 2013 and brought to the laboratory. Bolls were dipped for 20 seconds in the previous crude or purified protease and lipase separately or in mixture (v/v) and left to air dry under laboratory condition. Controls bolls were dipped in water only. One cotton bolls treated and one untreated were suspending in clean glass jars. As the same manner 5 replicates were repeated. Newly emerged moths of pink bollworm were sexed (male and female) and transferred to glass jars (three pairs /glass jar), the moths were fed on 10% honey solution. Eggs laid per female were counted on both enzyme treated and untreated bolls of each replicate (glass jar) after four days from the treatment. The percentage of repellency value was calculated according to the following equation of Lwande *et al*. (1985):

$$D = 1-(T/C) \times 100$$

where: T and C represent the mean number of eggs oviposited per females on treated and untreated bolls, respectively.
D = The percentage of repellency

b- Mortality effects of crude and purfied enzymes on the percentages of larval mortality, pupal mortality, adult emergency and deformed adult of pink bollworm

This experiment aimed to study the effects of crude and purified enzymes on some biological aspects of cotton bollworm *P. gossypiella*, mixing the diet (4 gm) with the enzymes of 4 ml for each dish. While, the diet of control mixed with water only. Each treatment was replicated five times. Batches of 20 newly hatched larvae each (0 - 6 h old) were transferred to the treated Petri-dishes after 30 minutes from treatment. Treated Petri -dishes were covered by fine and soft paper below the glass cover to prevent larvae escape. All treatments were incubated in the incubator running at constant conditions 26 ± 1ºC and 80 % relative humidity. After 24hr of exposure and feeding, dead and alive larvae were counted. The mortality percentages were estimated.

To study the latent effect of tested isolates on certain biological aspects of *P. gossypiella*, the survived larvae of each treatments were transferred individually to glass tubes (2 x 7.5 cm) containing 4 gm of untreated diet. Afterwards, glass tubes were covered with a piece of absorbent cotton and held under the same conditions as mentioned above. Larvae were examined daily to record the date of larval and pupal mortality. Also date of adult emergence and percentage of deformed adult were recorded El-Sayed (2008).

Statistical analysis

The obtained results of each mortality and biological parameters were subjected to analysis of variance to clear toxicity and latent effect parameters using Costat computer program Cohort Software. P. O. Box 1149, Berkeley CA 9471 (Costat statistic software, 2005).

Results and Discussion

Optimization of S. vinaceusdrappus SA protease and lipase production

The effect of environmental conditions on the production of proteolytic and lipolytic enzymes could play an important role in the induction or repression of the enzyme by specific compounds (Wang *et al.,* 2008). In the present investigation, the maximum protease production was achieved when *S. vinaceusdrappus* SA was cultivated in starch nitrate broth medium(pH7) with replacing of $NaNO_3$ by 2% gelatin (pH 7) at 30°C for 6 days in presence of 1% casein and 0.5% maltose. While, the maximum lipase production was achieved by incubation of tested organism in Dox-yeast extract-tributyrin broth medium (pH 7) at 30°C for 5 days in presence of 0.5 % yeast and 1% sucrose, under static conditions for both enzymes (Data not shown). These results were almost similar with Abou Zeid *et al.* (2007) for protease produced by *Streptomyces albidofiavus* Dakh-52 and Yu *et al*. (2007) for lipase produced by *Yarrowia lipolytica*.

Purification of S. vinaceusdrappus SA protease and lipase

The protease and lipase enzymes were purified as an extracellular enzymes from the liquid cultures of *S. vinaceusdrappus* SA growing under the optimal

culture conditions, by 70% $(NH_4)_2SO_4$. From the purification profile of the tested enzymes, the specific activities of protease and lipase were 116.7 and 104.9 U mg^{-1} protein compared to 8.4 and 8.9 U mg^{-1} crude protein, respectively. Also, the purification folds were 13.9 and 11.8 with 37 and 29% yield after sephadex-G50 for purified protease and lipase, respectively (Table 1). Similarly, the highly active alkaline protease from *B. licheniformis* MP1 was achieved after purified by Sephadex-G100 gel filtration (Jellouli *et al.*, 2011). Also protease from *Streptomyces* sp. A6 was purified to 34.56 fold by Sigh and Chhatpar (2011) using gel permeation chromatography. On the other hand, lipase of *S. thermocarboxydus* ME168 was purified to 9.5 fold with 20% yield following to precipitation by acetone and gel filtration chromatography using sephadexG-200 (H-kittikun *et al.*, 2012). Similarly, Bose and Keharia (2013) stated that the purity of lipase from *P. aeruginosa* AAU2 increased by 4.96 fold after ammonium sulfate precipitation followed by sephadex G-100 gel filtration chromatography.

TABLE 1. Purification steps of *S. vinaceusdrappus* SA protease and lipase.

Purification step		Total Activity (U)	Total Protein (mg)	Specific activity (U/ mg.protein)	Purification Fold	Yield %
Crude enzyme	Protease	152	18	8.4	1	100
Amm. Sulphate70%		112	8.5	13.2	1.6	73
Sephadex G_{100}		82	1.05	78.1	9.3	54
Sephadex G_{50}		56	0.48	116.7	13.9	37
Crude enzyme	Lipase	148	16.5	8.9	1	100
Amm. Sulphate 70%		102	7.6	13.4	1.5	69
Sephadex G_{100}		73	0.95	76.8	8.6	49
Sephadex G_{50}		43	0.41	104.9	11.8	29

In the present investigation, the homogeneity and molecular mass of each purified protease and lipase enzymes were studied. Each enzyme was appeared as a single protein band of 71 kDa (Fig. 1). This result is unique as only a few reporters are available on the halophilic thermostable protease having such a high molecular weight. Sookkheo *et al*. (2000) reported that the production of thermostable protease of molecular mass 71 kDa from *Bacillus stearothermophilus* strain TLS33 . Jain *et al*. (2012) detected the same molecular mass of protease produced from *Bacillus* sp. SM2014. Abou Zeid *et al*. (2007) recorded 60 and 35 kDa for alkaline protease of *S. halstedii* Salh-12 and *S. endus* Salh-40, respectively. While 21 kDa for *S. thermocarboxydus* ME168 lipase was detected by H-kittikun *et al.* (2012).

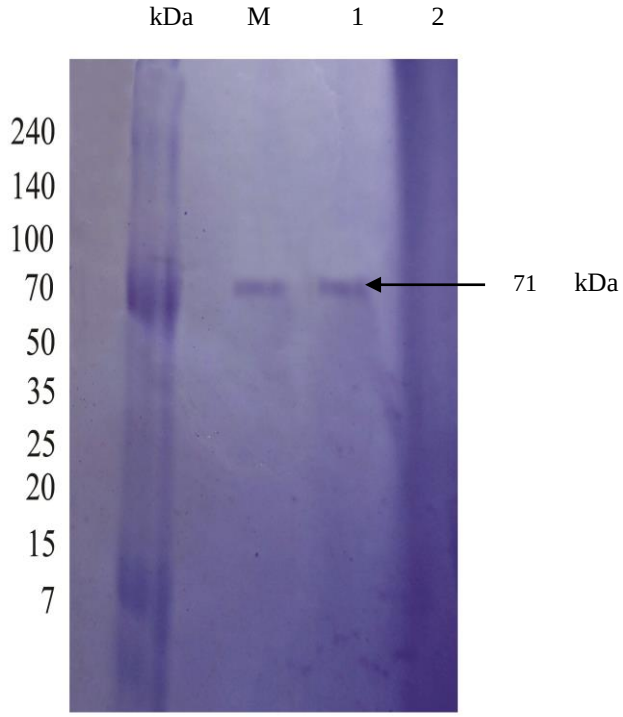


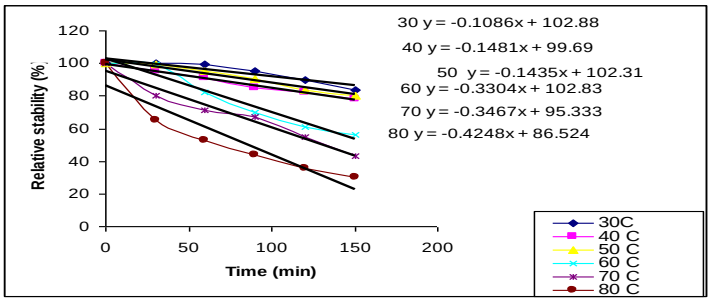
Fig. 1. SDS- PAGE of purified protease and lipase from *S. vinaceusdrappus*. Lane M (Broad-way Daul preslained protein marker), Lane 1, the purified protease enzyme; Lane 2, the purified lipase enzyme.

Biochemical and kinetic properties of S. vinaceusdrappus SA protease and lipase

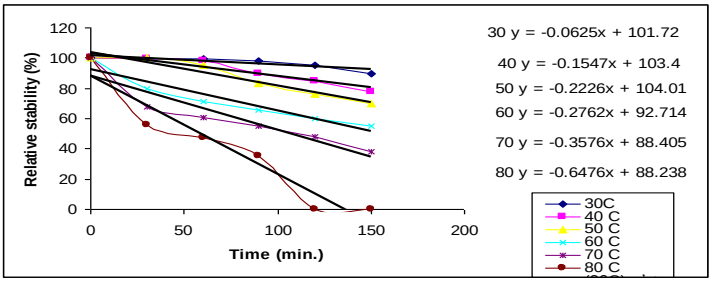
Concerning the biochemical properties of the purified protease and lipase, the maximum protease and lipase activity were at neutral pH 7 and 55 & 60°C respectively (data not shown). These results were agreed with Sigh and Chhatpar (2011). They clarified that the protease of *Streptomyces* sp. A6 exhibited highest activity at neutral to near-alkaline pH 7-9 and 55°C. Also, Jain *et al*. (2012) detected 60°C for maximum protease activity produced from *Bacillus* sp. Li *et al*. (2009) characterized an alkaline protease from *B. licheniformis* YPIA with optimum activity at pH 9.5 and 60°C. Also, H-kittikun *et al*. (2012) detected the maximum activity of lipase from *S. thermocarboxydus* ME168 at 50°C. Thermal and pH stability of the enzyme may be related to that the enzyme maintains its secondary and tertiary structure under stress but lowering of enzyme activity at high temperature or pH (above the maximum range) may be due to enzyme aggregation, incorrect structure formation, hydrolysis of S-S bonds, deamination and denaturation (Ahern and Klibanov, 1987).

Linear relation between the relative activity and reaction time of both enzymes at different temperatures (30, 40, 50, 60, 70, and 80°C) were observed

in (Fig. 2 a, b). It was found that, protease and lipase under investigation were expected to be inactivated above 80°C. Regarding thermal kinetic parameters, thermal inactivation rate (kr) and half-life time ($T_{1/2}$) decreased as temperature increased and the recorded half-life temperature Tm were 86.5 and 83.9°C for protease and lipase, respectively (Table 2) . Concerning this finding, Sigh and Chhatpar (2011) found that, the protease of *Streptomyces* sp. A6 was highly stable at temperature prevailing under field conditions (40°C) as apparent from kr and $T_{1/2}$ values 0.0065 and 106.75 min, respectively. H-kittikun *et al.* (2012) found that lipase of *S. thermocarboxydus* ME168 showed high stability at a broad pH and was thermostable at the temperature range 25-60°C with half life of 180 min at 65°C.



(a)



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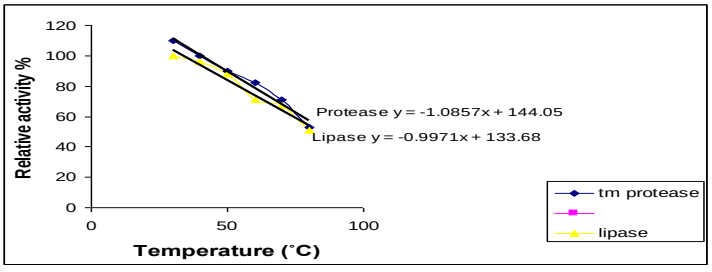


(c)

Fig. 2. Thermal stability profile of *S. vinaceusdrappus* for both enzymes, after incubation of enzymes at different temperature (30-80°C) and various periods (30-150 min), the residual activity was determined by the standard assay method (a) protease enzyme and (b) lipase; (c) Thermal inactivation profile for protease and lipase, Tm is temperature degree at which the enzyme retains half of its initial activity at 60 min.

TABLE 2. Thermal inactivation parameters of *S. vinaceusdrappus* SA on the activity of protease and lipase.

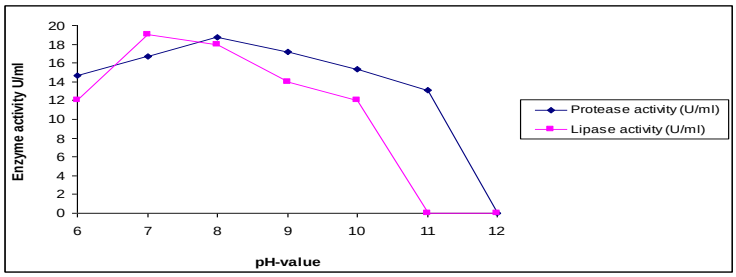
Temperature (°C)	Protease			Lipase		
	$T_{1/2}$ (hr)*	kr S^{-1}**	Tm*** (°C)	$T_{1/2}$ (hr)*	kr S^{-1}**	Tm*** (°C)
30	8.11	1.09×10^{-3}	86.5	13.8	6.28×10^{-4}	83.9
40	6.10	1.56×10^{-3}		5.8	1.57×10^{-3}	
50	5.59	1.47×10^{-3}		4.1	2.28×10^{-3}	
60	2.67	3.57×10^{-3}		2.6	3.29×10^{-3}	
70	2.17	4.09×10^{-3}		1.8	4.64×10^{-3}	
80	1.44	5.83×10^{-3}		0.98	9.69×10^{-3}	

* Half-life times ($T_{1/2}$) expressed by hours.

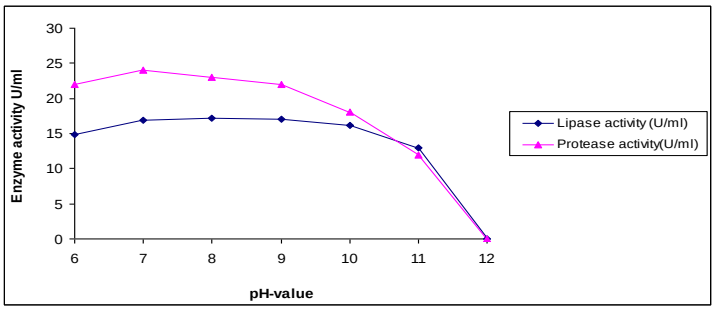
** Thermal inactivation rate (kr) expressed per seconds.

*** Half-life temperature (Tm) expressed by °.

Moreover it was found that the purified tested enzymes showed a wide range of pH stability. Data in (Fig. 3) showed that, the tested enzymes were approximately stable between pH 7.0 to 11.0 and the activities significantly decreased below and above these value. The present findings suggest that, the two enzymes are alkaline in nature. This result is almost similar with Zambare *et al*. (2011) and Bose and Keharia (2013) for protease produced by *P. aeruginosa* MCM B-327 and for lipase produced by *P. aeruginosa* AAU2, respectively.



(a)



(b)

Fig. 3. The pH value(a) and pH stability profile (b) of *S. vinaceusdrappus* protease and lipase.

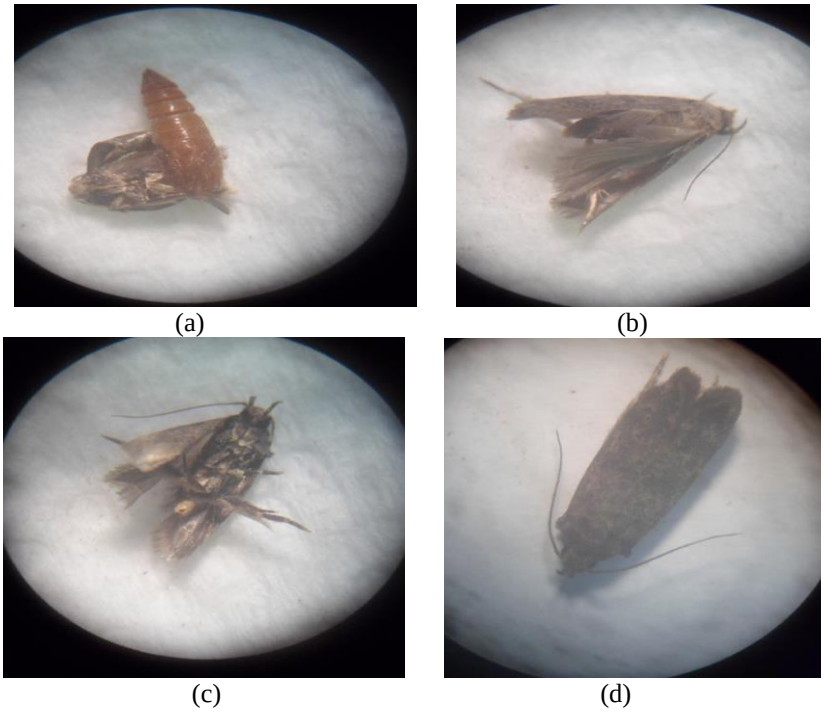


Fig. 3. Effect of treatment with purified *S. vinaceusdrappus* protease and lipase mixture (v/v) (a) Adult larvae cannot emerge from pupa due to treatment by the mixture of both pure enzymes; (b) Deformed adult of *Pectinophra gossypiella* treated with the purified mixture (c) Deformed adult of *Pectinophra gossypiella* treated with mixed purified enzymes, and (d) Control (without treatment).

Biocontrol of Pectinophra gossypiella using crude and purified S. vinaceusdrappus SA protease and lipase

a- Effect of S. vinaceusdrappus SA crude and purified enzymes on penetration of newly hatched larvae of pink bollworm in cotton bolls

The penetration of newly hatched larvae of pink bollworm into the cotton bolls after being dipped in different enzymes treatment are presented in (Table 3). The mixed purified enzymes treatment only 30% of the larvae were penetrated into the cotton bolls while penetration increased in presence of each purified enzyme alone. On the other hand, penetration percentage decreased in purified enzymes than crude enzymes. Also **,** the mixed crude enzymes penetration percentage decreased than the treatment of each crude enzyme alone. The highest successful penetration of newly hatched larvae of pink bollworm (60.00 %) was recorded with crude protease or lipase as compared with 80.00 % for control. Also, Elmedany (2013) detected the similar results on spiny bollworm when treated with garlic oil.

TABLE 3. Effect of crude and purified *S. vinaceusdrappus* SA protease and lipase on penetration of newly hatched larvae of *Pectinophra gossypiella* into cotton bolls.

Enzyme treatment		Alive larvae	Dead larvae	%Penetration
Crude	**protease**	6b	4b	60b
	lipase	6b	4b	60b
	Mixed	5bc	5ab	50bc
Purifie d	**protease**	4bc	6ab	40bc
	lipase	5bc	5ab	50bc
	Mixed	3c	7a	30c
Control		8a	2c	80a
F. test		n .s.	***	***
$LSD_{0.05}$		1.8947	1.6213	16.213

b- Repellent effect of S. vinaceusdrappus SA protease and lipase on pink bollworm moths

Data in (Table 4) showed that the tested enzymes had powerful repellent action for pink bollworm moths. It was found that, zero deposited eggs was observed when bolls were treated with the mixed pure protease and lipase as bolls compared with 49 eggs for control, resulting in 100.00 % repellent actions. While using purified protease or lipase alone**,** the treated bolls recorded 4, 5 deposited eggs as bolls compared with 56 and 55 eggs for control resulting in 92.9 and 90.26% repellant effect respectively. These results agree with the findings of Ahmed *et al*. (2007), They observed that the repellent effect of some oils on both pink boll worm moths and egg lying reduced significantly on the treated bolls.

Also data in the same table showed that crude protease, lipase and their mixture had repellent action for pink bollworm moths, where the treated bolls recorded 53, 49 and 51 deposited eggs as boll compared with 21, 19 and 2 eggs for control, resulting in 60.38, 61.64 and 81.05 % repellent actions respectively. These repellent actions may be due to bad odor and viscosity of purified enzyme since the moths can not lay eggs on the bolls. This result is almost in agreement with Wimalaratne *et al*. (1996). They found that the volatile extracts of pepper tree leaves *Schinus molle* have repellent activity against the house fly *Musca domestica*. Also, Hegab (2008) reported the repellent effect of Z-seed oil of Zanzalacht tree *Azadirchtaa indica* against the bollworms newly hatched larvae. Moreover, this results are similar to Elmedany (2013) using volatile oil on spiny bollworm**.**

TABLE 4. Repellent effect of crude and purified *S . vinaceusdrappus* SA protease and lipase of pink bollworm moths.

Enzyme treatment		Control	Treated	%repellent
Crude	**Protease**	48.6c	19b	61.64b
	Lipase	53ab	21a	60.38b
	Mixed	51bc	2e	81.05ab
Purified	**Protease**	56a	4d	92.8a
	Lipase	55a	5c	90.26a
	Mixed	48c	0f	100a
F. test		***	***	**
$LSD_{0.05}$		3.1657	1.1859	20.08

c- Mortality effects of S. vinaceusdrappus SA crude and purified enzymes on percent of larval mortality, pupal mortality, adult emergency and deformed adult of pink bollworm

1- Larval mortality percentage: Analysis of variance of the data given in Table (5) indicated highly significant effects between the enzyme treatments on larval mortality percentages of pink bollworm compared with control. The highest average percentage of larval mortality (75.0%) was recorded with the mixture of purified protease lipase (1:1v/v), whereas the lowest one (44.0%) was recorded for crude lipase as compared with control (4.66%).

TABLE 5. Effect of crude and purified *S. vinaceusdrappus* SA protease and lipase on larval, pupal and adult stages of pink bollworm *Pectinophra gossypiella*

Enzyme treatment		% of larval mortality	% of pupal mortality	% of adult emergence	% of deformed adult
Crude	**Protease**	46.3d	2.6a	93.33ab	2c
	Lipase	44d	1b	93.66ab	3bc
	Mixed	70b	3.2a	91.3b	4ab
Purified	**Protease**	55.6c	4.33a	90.3b	4ab
	Lipase	48d	4a	93 ab	5a
	Mixed	75a	4.3a	90.3b	5.6a
Control		4.66e	0.3	99a	0d
F- test		***	***	*	***
$LSD_{0.05}$		3.705	1.5756	4.4114	1.3778

*2- Pupal mortality percentage:*Data in (Table 5) showed that highly significant effect in pupal mortality percentages as compared with control. Mean pupal mortality percentages were 0.3 % in control, while 4.33% in mixture of purified enzymes and purified protease while the lowest effect on pupal mortality was 1.0% when using crude lipase against pink bollworm.

3- Adult emergence: Data in (Table 5) showed significant effects on adult emergence percentage as compared with control. Adult emergence percentages was 99.00 % with control, but the purified enzyme was effective more than the crude. The most effective treatments were purified protease enzyme and the mixture of purified protease and lipase. These treatments gave 90.3% of adult emergence.

4- Deformed adult percentage: Data in Table (5) showed highly significant effect on the deformed adult% compared with control since the purified enzyme had effective role than crude enzyme (Fig. 3). The most effective treatment was the of mixed purified enzymes (5.6%) while the lowest value was for crude protease (2%). The previous results are almost similar as shown by El Sayed (2008) who revealed a significant effect between the control and the treated *Pectinophra gossypiella* by protease enzyme produced from *Paecilomyces violacea* and *Paecilomyces varaioti*.

Mahfouz and Abou El-Ela (2011) mentioned that microbes could be used where chemical pesticides are banned. Also, chemical pesticides contaminate ground, water and enter food chains that have an impact on a wide range of organisms. While microorganisms produce several cuticle degrading enzymes used for biological control as protease on *Pectinophra gossypiella* (El-Sayed, 2008), chitinase on *Callosporuchus maculatus* (Ghareeb, 2009) and lipase against *Rhopaosiphum maidis* (Lokma, 2010).

Microorganisms produce a cuticle degrading enzymes particularly proteases and lipases on the surface of host shell during the infection process (Sahebani and Hadavi, 2008). The microorganisms able to germinate on insect cuticle directly, then secrete complex group of enzymes including protease and lipase to dissolve the cuticle barriers and penetrate the insect haemocoel (Gillespie *et al*., 1998; Pedrini *et al*., 2006 and El-Sayed, 2008).

Conclusion.

S. vinaceusdrappus SA (AB857336) had ability to produce protease and lipase. The purified enzymes showed broad pH stability and high thermal stability. Also, the crude and purified enzymes are being promising candidates for application as biological control agents to control pink bollworm *Pectinophra gossypiella.*

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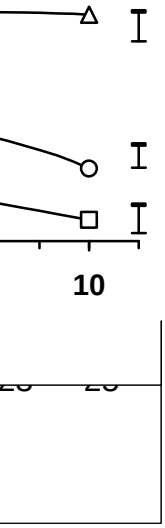

المكافحة البيولوجية لدودة اللوز القرنفلية (سوندرز) بواسطة إنزيمى البروتييزو الليباز المنتجين من استربتوفينادرابس

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تم تحضين كائن استربتوفينادرابس تحت الظروف المثلى ، في درجة الحموضة 7.0 عند 30 درجة مئوية فى وجود 1 ٪ من الكازين و 5. . ٪من المالتوز و 0.5 ٪ من الخميرة و 1 ٪ من السكروز لمدة 6 و5 أيام 5 للبروتياز و الليباز ، على التوالي . أجريت التنقية الجزئية لانزيم البروتييزوالليباز بكبريتات الأمونيوم ، ثم باستخدام سيفادكس ج 100 يليه سيفادكس ج 50 . و بعد خطوة التنقية النهائية ازداد نشاط البروتياز و الليباز الى 116.7 و104.9 ملجرام بروتين مقارنة ب 8.4 و 8.9 ملجرام من البروتينات الخام ، مع العائد 37 و 29 ٪ ، على التوالي . وكان الوزن الجزيئى للإنزيمين بعد التنقيه هو 71 كيلو دالتون . أيضا ، كان على نشاط للإنزيمين عند الرقم الهيدروجيني 7.0 مع ثبات فى النشاط من 7،0 حتي 11،0 اس هيدروجيني. وكان أعلى نشاط للإنزيمين عند درجتى حرارة 55 و 60 درجة مئوية و 86.5 Tm و 83.9 درجة مئوية للبروتييز و الليباز ، على التوالي . وبدراسه قدره اليرقات حديثة الفقس من دودة اللوز القرنفلية للقطن لاختراق لوز القطن بعد معلملتها بالبروتياز و الليباز (الخام و النقي) كل على حدا او معا (حجم\حجم) اظهرت النتائج أن الزيادة فى نسبة الاختراق المئوية في وجود الإنزيمات الخام أكثر من النقية . و انخفضت نسبة الاختراق المئوية في وجود الإنزيمات الخام المختلطة أكثر من وجود كل إنزيم على حدة . أيضا ، وكان الليباز و البروتياز الخام و خليط منهم له عمل طارد لفراشات دودة اللوز القرنفلية. وان هناك تأثير كبير للغاية على نسبة الفراشات البالغة المشوهة مقارنة مع الفراشات الغير معاملة و كان للإنزيم المنقى دور فعال وأكثر تأثيرا من الانزيم الخام .