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Effect of Some Lytic Enzymes Produced by *Streptomyces sp.* on the Camel Tick *Hyalomma dromedarii* Eggs (Acari: Ixodidae)

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Abstract

THE lethal and inhibitory effect of the chitinase and protease enzymes supplied by *Streptomyces* sp. NRC23; NRC16; NRC18; NRC12; NRC80; NRC 90; NRC 50; *Streptomyces gisens* NRRL2021 and *Streptomyces pseudo griseolus*, on *Hyalomma dromedarii* eggs were evaluated. Five concentrations from chitinase and protease enzymes were prepared. The concentrations ranged from 1/1 to 1/5 (ml enzyme / ml distilled water) were tested at room temperature. The results were obtained seven days after the treatment of the eggs. Except for *Streptomyces* sp. NRC23, the chitinase enzyme had the greatest inhibitory effect across all types of *Streptomyces* species. The lethal effect was higher than the inhibitory effect on *H. dromedarii* eggs. *Streptomyces* NRC12's chitinase enzyme had a greater inhibitory effect. The number of dead eggs treated with *Streptomyces sp*. NRC23 chitinase enzyme was 49±0; 40±3; 40±7 eggs at concentration 1/2; 1/3; 1/5(v/v), respectively. The number of inhibited eggs treated with chitinase enzymes of *Streptomyces sp*. NRC23; *Streptomyces sp*. NRC16; *Streptomyces* NRC18; *Streptomyces* NRC12 and *Streptomyces gresius* NRRL2021 were 36±8; 31±1; 48±1 and 38±8 eggs at concentration 1/2; 1/1; 1/3 and 1/1 (ml/ml), respectively. The lethal effect on eggs treated with *Streptomyces pseudogriseolus* protease enzyme was 39±3 eggs at concentrations 1/1; it's higher than all types of *Streptomyces*. Inhibitory and lethal effects on eggs treated with protease enzyme produced by *Streptomyces* sp. NRC 90, *Streptomyces sp* NRC 50, and *Streptomyces* sp. NRC80 were 39±5, 27±9, 31±2 eggs and 20±6, 25±11, 32±6 eggs, at concentrations 1/3, 1/4, 1/2 and 1/2, 1/3, 1/1 (ml/ml).

Keywords: Control, Inhibition, Lethal, chitinase, protease

Introduction

Actinomycetes are gram-positive bacteria mycelium-forming soil. [1]. They manufacture lytic enzymes which destroy various macromolecules as cellulose, chitin, proteins, lignin xylan, starch, lipids, keratin, and pectin. [2]. Also, they can produce antibiotics and commercial compounds [3]. Actinomycetes are used as biocontrol as antifungal compounds, and biopesticide agents [4]. Insecticidal activity found in both actinomycete cells and cell filtrate could be used against insect pests. The positive effects of actinomycetes and their metabolites have been well assessed [5]. Among many actinomycetes, the genus *Streptomyces* is always of particular interest in research since it produces a wide range of chemicals with diverse biological features. In Egypt, actinomyces strains were used as a control agent against the number of agriculture and medical insects such as *Galleria mellonella, Drosophila melanogaster*; house fly *Musca domestica* and 3rd instar larvae of mosquitoes *Culex pipiens* [6,7]. Additionally, actinomyces strains were used as biocontrol agents against internal parasites of *Fasciola gigantica* eggs and *Toxocara vitulorum* [8,9]. Actinomyces positive chitinase *Streptomyces rochei*; *Streptomyces minutiscleroticus; Streptomyces phaeoluteigrisseus* and *Streptomyces cacaoi* sup sp. were used against 3rd instar larvae of mosquitoes *Culex quinquefasciatus*; *Aedesa egypti* and Red flour beetle *Tribolium castaneum* [10].

Several studies have focused on biological control as an alternative strategy to eradicate tick infestation [11, 12]. Chitinase enzyme that was extracted from cell walls of *Candida albicans* and

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Aspergillus fumigates from soil was used against the cattle tick *Boophilus microplus* [13]. Protease and chitinase enzymes produced by environmentally safe soil fungal species could be employed to biocontrol the camel tick *H. dromedarii* eggs to eliminate the need for chemicals control. [11]

This study is a complement part of a previous work published in [11]. The influence of chitinase and protease enzymes produced by environmental isolations of *Streptomyces* species on the camel tick *H. dromedarii* eggs were shed lighted.

Material and Methods

Microorganism

Isolation: Soil samples were collected from two villages; Zawyet Ghazal, (*31.0425° N, 30.4728° E*) in Damanhur (El Beheira governorate) and Abshoway (29.35805° *N,* 30.68142° *E*) in El Faiyum governorate. Furthermore, seawater samples were collected from El Obayed beach (31.3703° *N*, 27.0934° *E*) in Matrouh governorate and El Hurghada beach (27.2582° *N*, 33.8123° *E*) in Red Sea governorate. The samples were diluted in a sterile saline solution (0.89% w/v) before being transferred onto sterile starch nitrate plates, containing (g/l) Starch 20; K2HPO4 0.5; KNO3 1; Fe SO4 0.01; MgS047H20 0.5; NaCl 0.5; and agar 20 ,and adjusted at pH 7.0 were incubated at 30°C [14]. After seven days of incubation, the growing actinomycetes and fungal colonies were isolated and subcultured onto fresh plates. Single colonies were then transferred into slants containing the same medium. These slants were stored in a refrigerator at 4°C until they were needed.

To identify bacteria capable of producing chitinase, Luria-Bertani medium (LB) is commonly used. However, in this specific case, a different medium was chosen to specifically promote the growth of chitinase-producing microorganisms. Typically, when the strain is grown on a medium containing beef extract or peptone, it will exhibit a red colony.

The selective medium used in this process contained 1% colloidal chitin (w/v),0.5% (NH4)2SO4 (w/v), 0.05% MgSO4_7H2O (w/v), 0.24%KH2PO4 (w/v), 0.06% K2HPO4 (w/v), pH 7.0[15]. LB medium, contained 1% peptone (w/v), 1% beef extract (w/v), 0.5% NaCl (w/v), pH 7.0. Solidified LB medium was from adding 1.5% of agar (w/v) into LB medium. The results of these tests provide valuable information about the strain's biochemical profile, which aids in its identification. By comparing the test results with known profiles of microorganisms, researchers can narrow down the potential species or genus to which the isolated strain belongs in (Microbial Chemistry Dept.).

Screening of some Streptomyces for production of chitinolytic and proteolytic activities

Chitinase production

The basal medium used for the optimization studies contained (g/l): KH_2PO_4, 3.0; K_2HPO_4, 1.0; $MgSO_4$, 0.7; $(NH_4)2SO_4$, 1.4; NaCI, 0.5; $CaC1_2$, 0.5; yeast extract,0.5; bacto-peptone, 0.5 and chitin, 5.0. The pH of the medium was adjusted to a range of 6.5-7.0 before it was autoclaved. The experiments were conducted using shake flasks, with each flask containing 100 ml of medium in a 500-ml Erlenmeyer flask. The flasks were incubated at 28°C with continuous shaking at 150 rpm for a duration of 7 days. For further investigations, a specialized medium optimized for enzyme production, as described by [10], was utilized. In all experiments, spore inoculums from 7-day-old slants were used, with a quantity of 10' per flask.

Chitinase assay

Chitinase activity was assessed using the dinitro-salicylic acid (DNS) method [15]. This method relies on measuring the concentration of N-acetyl glucosamine (NAG) released due to enzymatic action [16,17]. To determine chitinase activity, a 2-ml reaction mixture was prepared. It consisted of 1 ml of 0.1% colloidal chitin in acetate buffer (50 mM, pH 5.0) and 1 ml of crude enzyme extract. The mixture was then incubated in a water bath shaker at 50 °C for 1 h. After incubation, the reaction was halted by adding 3 ml of DNS reagent to 1 ml of the filtrate. The mixture was heated at 100 °C for 5 minutes, and the absorbance was measured at 540 nm using a UV spectrophotometer. Enzyme activity was quantified based on the amount of enzyme required to catalyze the release of 1 µmol of N-acetylglucosamine per ml within 1 minute. This unit of enzyme activity is defined as one unit. The colloidal chitin used in the experiment was prepared according to the method described by [18].

Protease production

The culture was inoculated into 250 ml Erlenmeyer flasks containing 100 ml of production medium. The production medium consisted of glucose (150 mg), K2HPO4 (20 mg), KH2PO4 (20 mg), MgSO4 (10 mg), CaCl2 (10 mg), casein (200 mg), and NaNO3 (100 mg), with a pH of 8.5. The flasks were placed on an environmental shaker at 150 rpm and incubated at 28 ºC for a period of 5 days to allow for enzyme activity to develop. After incubation, the supernatant was collected by centrifuging the mixture at 15,000 rpm and 4 ºC for 15 min. The resulting supernatant was used as crude enzyme [19].

For the protease assay, the method described by [20] was followed. In brief, 3 ml of the crude enzyme was mixed with 3 ml of citrate phosphate buffer and 3 ml of 1% (w/v) casein in a 25 ml test tube. The tube was then placed in a water bath at 35 °C for 1 hour to allow the enzyme-substrate reaction to occur. To stop the reaction, 5 ml of 20% (w/v) trichloroacetic acid (TCA) was added. After one hour, the solution was filtered using Whatman grade 540 (ashless) filter paper. From the filtrate, 1 ml of the enzyme-substrate mixture was transferred to a test tube, and 2 ml of 20% Na2CO3 was added. To this mixture, 1 ml of Folin-Ciocalteu reagent was added, and the contents of the tube were immediately mixed well. After 30 minutes, 6 ml of distilled water was added to the tube, and the absorbance of the solution was measured at 650 nm using a Vis-UV spectrophotometer (LaboMedInp). The amount of amino acids released was determined by referencing a standard curve plotted from known concentrations of tyrosine. Enzyme activity was expressed in units, with one unit defined as the amount of enzyme capable of releasing 1 g of tyrosine from the substrate (casein) per hour under the assay conditions.

Preparation of dead enzyme

The crude culture filtrate was autoclaved at 121°C, 1.5 atm for 5 minutes.

Ticks

Engorged females of *H. dromedarii* were collected from the ground of camel market in Burkash village, Giza governorate, Egypt. Identification of females was confirmed in the laboratory according to [21]and [22]**.** Females were incubated at a constant temperature of 24 ± 2̊ C with a relative humidity of 75 ± 5 % in permanent darkness to obtain eggs [23]. One to seven-day old eggs were used in the experimental assays [11].

Inhibitory and lethal activity of chitinase and protease enzymes

The concentrations of enzymes derived from *Streptomyces* sp. *NRC23*; *Streptomyces sp. NRC16*; *Streptomyces* sp. *NRC*18; *Streptomyces* sp. *NRC 12*; *Streptomyces gisens* NRRL2021; *Streptomyces sp NRC80*; *Streptomyces* sp *NRC* 90; *Streptomyces* sp NRC 50 and *Streptomyces*

pseudogriseolus were prepared by using distilled water. The five concentrations were prepared [crude enzyme: distilled water (v/v)] ranged from 1/1 to 1/5 ml of enzyme / ml distilled water. In control treatment the distilled water was used alone for each concentration. The concentrations of the nine stock solutions from which these dilutions were prepared had enzyme concentration of 4 units/ml. Each concentration or control treatment was replicated 3 times, each replicate included 50 healthy eggs. The test was applied by dipping healthy eggs for 50 second in 200µl from each concentration dilution or distilled water in control treatment and left to dry and then incubated at room temperature until hatchability occurred. Mortality calculated of eggs were based on eggs with brown-black color and abnormal shape and corrected by Abbott's formula [24]. Inhibited eggs were counted after seven days of the treatment (None of which reached the hatching stage). Normal eggs have oval shape and shiny brown in color, and were left to develop until hatching occurred.

Statistical analysis

Statistical analysis of data, including the calculation of mean and standard deviation (SD) were done. One-way analysis of variance followed by Duncans multiple range test was used for the significance differences between treated groups. Differences were considered significant at P<0.05 level [25]. SPSS version 10 computer program download from http://www.Spss.com.

Results

The growth and chitinase; protease production of *Streptomyces* sp. are illustrated with *Streptomyces* sp. NRC 23; *Streptomyces* sp. NRC 16; *Streptomyces* sp.NRC 18; *Streptomyces sp.*NRC 12 and *Streptomyces gresius* NRRL2021 on media as a qualitative test and *Streptomyces* sp. NRC80; *Streptomyces* sp. NRC 90;*Streptomyces* sp. NRC 50 and *Streptomyces pseudogriseolus* on media as a quantitative test (Tables 1, 2).

Inhibitory and lethal effect of chitinase and protease enzymes

Results are illustrated with (Table 3, 4). The lethal and inhibitory effect of chitinase and protease enzymes produced by *Streptomyces* sp. on Camel tick *Hyalomma dromedarii* eggs were studied. The results were obtained seven days after the treatment of the eggs.

Chitinase enzymes

The results showed that the inhibitory effect was highest in all types of *Streptomyces* species, except the *Streptomyces* sp. NRC23, the lethal effect was stronger than the inhibitory effect on *H. dromedarii* eggs (Table 3). Inhibitory activity of chitinase enzymes of *Streptomyces sp*.NRC23 were 41±8 and 23±28 at concentrations 1/1 and 1/3(v/v), respectively. On the other hand, the lethal effect of *Streptomyces sp*.NRC23 chitinase enzymes were 49±0; 40±3; 40±7 at concentrations1/2; 1/3; 1/5(v/v), respectively. But in concentration 1/4(v/v), the lethal effect was 24±2 (Table 3). The lethal effect of *Streptomyces sp*. NRC16 chitinase enzymes

fluctuates between 13±8 and 23±1 at concentrations 1/1 - 1/5(v/v) respectively. The higher inhibitory effect of *Streptomyces sp*. NRC16 chitinase enzymes was found in concentrations 1/2(36±8); 1/5(28±6); 1/3(26±2), and 1/4(25±1), (Table 3). Inhibitory effect of *Streptomyces* NRC 18 chitinase enzymes were 31±0; 25±5; 20±4; 20 and 19±9 at concentrations 1/1-1/5 ml/ml. The results of the lethal effect of *Streptomyces* NRC 18 chitinase enzymes illustrated that, 16±2; 14±2; 30±4; 21±6 and 29±9 at concentrations 1/1; 1/2; 1/3; 1/4 and 1/5 ml/ml respectively (Table3). *Streptomyces* NRC12 chitinase enzymes showed strong inhibitory effect (48±1and 32±7) at concentrations 1/3 and 1/4 respectively, but at concentrations 1/2; 1/1; 1/5 and 1/4 was 34±6; 29±6; 28±8 and 18±9 (table 3). The highest effect of chitinase enzymes of *Streptomyces gresius* NRRL2021 was 33±8; 31±4 and 12±6;18±5 at concentrations of 1/1;1/3 and 1/1;1/4 in inhibitory and lethal effect respectively (Table 3).

Protease enzymes

In this study, protease enzymes showed the differences between the *Streptomyces* sp in inhibitory and lethal effect on *H. dromedarii* eggs tick. Inhibitory and lethal effect of *Streptomyces* sp NRC80 protease enzymes were 18±7; 29±5; 17±1; 28±5; 31±2 and 32±6; 29±5; 29±4; 13±6 and 24±11 at concentrations 1/1; 1/2; 1/3; 1/4 and 1/5, respectively. Inhibitory and lethal dose effect of the protease enzymes produced by *Streptomyces* sp NRC90**;** *Streptomyces* sp NRC50 and *Streptomyces pseudo griseolus* ,were 39±5; 27±9; 33±7 and 20±6; 25±11; 39±3 at concentrations 1/3; 1/4; 1/2 and 1/2; 1/3; 1/1, respectively (Table 4) .

Discussion

Chitinase producing actinomycetes from the soil of *Avicennia marina* -mangrove environment of Ariyankuppam M20 was selected to control the growth of larvae of mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*[10]. He said that chitinase-producing actinomycetes have a very high potential to inhibit chitin synthesis in insects. Chitinase and protease enzymes produced by some soil fungi were used as a bio acaricide against camel tick *Hyalomma dromedarii* eggs [11] . They found that chitinase and protease enzymes produced by some soil fungi have a marked ovicidal effect on *H. dromedarii* eggs. In this study, chitinase enzymes produced by *Streptomyces sp*. NRC16; *St*. NRC18; *St*. NRC12 and *St. gresius* NRCL2021 have an inhibitory effect on *H. dromedarii* eggs in agreement with Janaki [10] who said that chitinase produced by actinomycetes inhibits chitin synthesis in insects. Based on this, it is thought that the chitin enzyme has led to incomplete fetal development within tick eggs since it has inhibited chitin synthesis. Therefore, *H. dromedarii* eggs have not been hatched. The inhibitory effect of the enzyme was observed by monitoring the change in the shape of eggs from an oval shape to an abnormal shape (flattened with corrugated shell). The lethal effect of the enzyme was observed within the eggs that have a brown-black color and abnormal shape in line with [26] and [11]. They found that the complete death of eggs of *H. dromedarii* is based on its brown-black color and abnormal shapes (corrugated oval shape).

Lytic enzymes such as α and β-glucanases, proteases, peptidases, cellulases, chitinases, and lipases have been proposed as the key enzymes in the lysis of pathogenic bacterial and fungal cell wall, extracellular enzymes from entomopathogenic fungi, including proteases have been identified in the infection of the arthropod by pathogenic organisms[27] . Chitinase can also be used directly as biopesticides against various fungi and insects that can be an alternative to chemical pesticides[28,29]. Proteases known as proteinases or proteolytic enzymes, which occur naturally in all organisms, act on the peptide bonds formed by specific amino acids to hydrolyze them [27]. In the present study, protease enzymes are supplied from *Streptomyces* Sp. NRC 80, *Streptomyces sp*. NRC 90**;** *Streptomyces sp*. NRC 50 and *Streptomyces pseudo griseolus* NRRL 2021, have an ovicidal effect on *H. dromedarii* eggs. Results of this study are supported by Tunga et al. [27] and Habeeb et al. [11] who found that chitinase and protease enzymes produced by some soil fungi have an ovicidal effect on *H. dromedarii* eggs tick .

Conclusion

Chitinase and protease enzymes produced by all *Streptomyces sp*. had a potential ovicidal effect on *H.* eggs. *Streptomyces sp. NRC23*, chitinase enzyme revealed the greatest lethal effect and *Streptomyces* NRC 12 gave the greatest inhibitory effect on the camel tick *H. dromedarii* eggs. *Streptomyces pseudogriseolus* and *Streptomyces* Sp NRC 90 protease enzyme had the greatest lethal and inhibitory effect, respectively on *H. dromedarii* eggs. Chitinase and protease enzymes may play as an alternative to chemical pesticides.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. In- vitro growth on medium containing Casein for protease or chitin for chitinase.

Strain	Chitinase	Protease
Streptomyces sp. NRC18	++	-
Streptomyces sp. NRC90	++++	+++
Streptomyces sp. NRC50	-	+++++
Streptomyces gresius NRRL2021	+	+
Streptomyces pseudo griseolus	-	+++++
Streptomyces sp. NRC23	+++	-
Streptomyces sp. NRC16	+++	-
Streptomyces sp. NRC12	++	-
Streptomyces sp. NRC80	-	+++++

- No growth, + Weak growth, ++ Moderate growth, +++ Heavy growth, ++++ Very growth, +++++ Vigorous growth

TABLE 2. Production of chitinase and protease by Streptomyces

Strain	Chitinase activity(units/mg protein)+SD	Protease activity(Units/mg protein)+SD
Streptomyces sp. NRC18	835.7± 2.5	0.0
Streptomyces sp. NRC 90	1336.9±2.55	977.6± 2.7
Streptomyces sp. NRC50	0.0	2309± 2.7
Streptomyces gresius NRRL2021	490±1.0	11.3±1.0
Streptomyces pseudo griseolus	0.0	9859± 6.4
Streptomyces sp. NRC23	1211.12±8.33	0.0
Streptomyces sp. NRC16	1528.9± 3	0.0
Streptomyces sp. NRC12	893.91±5.8	0.0
Streptomyces sp. NRC80	0.0	2997.3±3

TABLE 3. Effect of chitinase enzymes of *Streptomyces Sp. nrc23; Streptomyces Sp.nrc16; Streptomyces nrc 18; Streptomyces nrc 12 and Streptomyces gresius NRRL2021* on the camel tick *Hyalomma dromedarii* eggs.

conc. ml /ml	*Streptomyces* Sp.nrc23 Mean±SD			*Streptomyces* Sp.nrc16 Mean±SD			*Streptomyces* nrc 18 Mean±SD			*Streptomyces* nrc 12 Mean±SD			*Streptomyces gresius* NRRL2021 Mean±SD		
	Hatch	Dead	Inh	Hatch	Dead	Inh	Hatch	Dead	Inh	Hatch	Dead	Inh	Hatch	Dead	Inh
1:1	0	8±8.33c	41±8.33a	5±0a	22±0.33	22±0	2±2.0b	16±2.66	31±0.66	5±4.04	29±6.88	15±2.90b	4±2.84	12±6.35	33±8.14
1:2	0	49±0.33a	0.3±0.33c	0±0b	13±8.33	36±8.33	11±3.21a	14±+2.00	25±5.13	1±0.88	34±6.76	13±5.89b	27±1.52	6±6.33	16±6.00
1:3	2+1	40±3.33ab	7±2.33bc	1±0.88b	22±2.66	26±2.96	0b	30±4.04	20±4.04	0	1±1.66	48±1.66a	17±6.11	1±1.66	31±4.48
1:4	1+1.66	24±2.40bc	23±3.28b	0.6±0.66b	23±13.69	25±13.22	0b	21±6.35	20±6.35	3±0.57	18±9.52	32±7.00ab	23±9.87	11±4.50	15±9.24
1:5	0.3+0.33	40±7.88ab	9±8.0bc	0±0b	21±5.81	28±6.11	2±2.0b	29±9.86	19±9.86	3±2.51	28±8.14	19±8.56b	19±5.81	18±5.66	12±3.60
F	1.186	8.985	8.996	7.818	0.294	0.513	5.727	1.585	0.821	0.720	3.340	6.292	2.087	1.529	2.128
P	NS	0.002	0.002	<0.001	NS	NS	0.012	NS	NS	NS	NS	0.009	NS	NS	NS

Mean±SD mean of eggs number and stander error, hatch: mean value of hatched egg mas in replicates, dead: mean value of dead eggs, inh: mean value of inhibted eggs after seven day of treatment. coc: concentration of enzymes. Different small letters in the same column represent significant differences between concentrations ($P < 0.05$). NS: non-significant

TABLE 4. Effect of protease enzymes of Streptomyces Sp NRC80; *Streptomyces Sp NRC 90;Streptomyces Sp NRC 50* and *Streptomyces pseudo griseolus* on the camel tick *Hyalomma dromedarii* eggs.

Cnoc.	*Streptomyces* Sp NRC80 Mean±SD			*Streptomyces* Sp NRC 90 Mean±SD			*Streptomyces* Sp NRC 50 Mean±SD			*Streptomyces pseudo griseolus* Mean±SD		
	Hatch	Dead	Inh	Hatch	Dead	Inh	Hatch	Dead	Inh	Hatch	Dead	Inh
1:1	0	32±6.65	18±66.5	1±0.57b	18±0.33	30+0.33	10±10	16±9.52	23±5.23	3±0.66	39±3.33	7±3.71b
1:2	0	29±5.20	29±5.20	0.6±0.66b	20±6.83	28±6.17	13±7.26	24±7.44	12±6.48	3.6±0.66	13±7.79	33±7.23a
1:3	3±3.33	29±4.66	17±1.33	0b	10±5.20	39±5.20	2±1.45	25+11.17	22±11.13	12±11.83	29±40.78	8±4.17b
1:4	6±4.48	13±6.17	28±4.93	3±0.57a	19±7.02	28±7.50	6±6	17±7.0	27±9.84	9±6.55	16±4.04	25±8.50ab
1:5	21±13.86	24±11.06	31±2.02	0.3±0.33b	18±2.30	31±2.20	21+10.96	14+7.02	15+4.35	0.6±0.66	29±3.48	20±2.88ab
F	1.701	1.053	2.819	5.682	0.625	0.869	0.825	0.365	0.007	0.609	2.634	3.621
P	NS	NS	NS	0.012	NS	NS	NS	NS	NS	NS	NS	0.045

Mean±SD mean of eggs number and stander error, hatch: mean value of hatched egg mas in replicates, dead: mean value of dead eggs, inh: mean value of inhibted eggs after seven day of treatment. coc: concentration of enzymes. Different small letters in the same column represent significant differences between concentrations ($P < 0.05$). NS: non-significant

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التأثير الإبادي لبعض الانزيمات المحللة التى تنتجها بكتيريا الأستربتو ميسيس على بيض قراد الجمل هيالوما دروميدارى (أكارى: إكسوديدى)

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الملخص

يوجد حوالي 800 نوع من القراد المنتشره في جميع أنحاء العالم حيث أنها وسيله لنقل بعض أنواع الفيروسات والبكتريا والبروتوزوا إلى جسم العائل. ويعتبر الهيالوما دروميداري واحد من تلك الأنواع التي تصيب الإبل في مصر. تستخدم المبيدات الكيميائيه على نطاق واسع فى مقاومه الطفيليات الخارجيه والداخليه وهى ذو كفاءه عاليه فى ذلك الا انها قد تؤثر بالسلبيه على التكاثر فى الحيوان وبالتالى الثروه الحيوانيه و لذلك كانت الحاجه ملحه لاستخدام مبيدات بديله طبيعيه امنه على صحه الحيوان وصديقه للبيئه. ركزت العديد من الدراسات على المكافحة البيولوجية كوسيله بديلة للقضاء على غزو القراد وتجنب استخدام المكافحة الكيميائية. تم في هذا البحث دراسة التأثير الأبادي والمثبط لإنزيمات الكيتينيز والبروتييز التي تنتجها أنواع مختلفه من بكتريا استربتوميسيس الامنه على الحيوان والبيئه; وهى *Streptomyces* sp. NRC16; *streptomyces* sp. NRC23 *Streptomyces sp*. NRC18; Streptomyces sp. NRC12; *Streptomyces gisens* NRRL2021; *Streptomyces* sp. NRC 50 ;*Streptomyces* sp. NRC 90 ;*Streptomyces* sp. NRC80 و*Streptomyces* *pseudo griseolus*على بيض قراد الجمل *Hyalomma dromedarii*. تم تحضير خمسة تراكيز من إنزيمى الكيتينيز والبروتييز وهى من 1/1 إلى 5/1 مل/مل عند درجة حرارة الغرفة على بيض قراد *Hyalomma dromedarii* بعمر البيض 7 أيام. أوضحت النتائج بعد اليوم السابع من المعامله بالمقارنه بالمجموعه الضابطه. التأثير التثبيطي لإنزيم الكيتينيز هو الأعلى في جميع أنواع الستربتوميسيس باستثناء *Streptomyces sp. NRC23*، كان التأثير المميت أعلى من التأثير المثبط على بيض قراد الجمل *Hyalomma dromedary*. بالإضافة إلى ذلك، كان التأثير التثبيطي لإنزيم الكيتينيز لبكتيريا *Streptomyces NRC12* أعلى من جميع أنواع الستربتوميسيس قيدهذه الدراسة. التأثير المميت لإنزيم الكيتينيز لبكتيريا *NRC23 Streptomyces sp*. كان ± 49؛ 7 ±40 ± 3؛ 40 عند التركيز 2/1؛ 3/1؛ 5/1 (حجم / حجم). كان التأثير التثبيطي لإنزيمات الكيتينيز المنتج بواسطه بكتيريا الستربتوميسيس بأنوعها وهى *St NRC23* - *St NRC16* - *St NRC*18 -*St* 12 *NRC* و استربتو ميسيس جريسيس *St.NRC2021* هى **8± 41 ؛ 8 ± 36؛ 1 ± 31؛ 1±48 و8±33**عند تركيز 1/1؛ 1/1؛2/1؛ 3/1 و1/1 (ت/ت). كما أظهرت هذه الدراسة، أظهر التأثير المثبط والمميت لإنزيم البروتييز للأنواع المختلفه لبكتيريا *Streptomyces sp*. في على بيض قراد *Hyalomma dromedarii*. كان التأثير الأعلى من جميع أنواع الستربتوميسيس للجرعة المميتة للإنزيم لنوع بكتيريا*Streptomyces pseudo* هى**3.33±39** عند التركيز 1/1. بينما كان تأثير الجرعة المثبطة لإنزيم البروتييز المنتج بواسطة بكتريا *Streptomyces sp. NRC 90* و *Streptomyces sp NRC 50* و *Streptomyces sp NRC80* هى *5 ± 39* و 9± 27 و 2± 31عند تركيزات 1/3 -1/4 - 1/5 و الجرعة والمميتة لنفس انواع البكتيريا هى 20 ± 6 و 25± 11 و 32 ± 6 عند تركيزات 1/2 -1/3 - 1/1. أوضحت النتائج أن الإنزيمات المحلله المنتجه بواسطه جميع أنواع بكتيريا *Streptomyces sp*. لها تأثيرات مثبطة وابادیه لنمو وتطور مراحل بيض قراد الجمل *Hyalomma dromedary*.

الكلمات الدالة: الإنزيمات محلله - بيضالقراد، *Hyalomma dromedarii* عوامل بيولوجيه - -مصر - الجمال - *Streptomyces* – بيكتيريا.