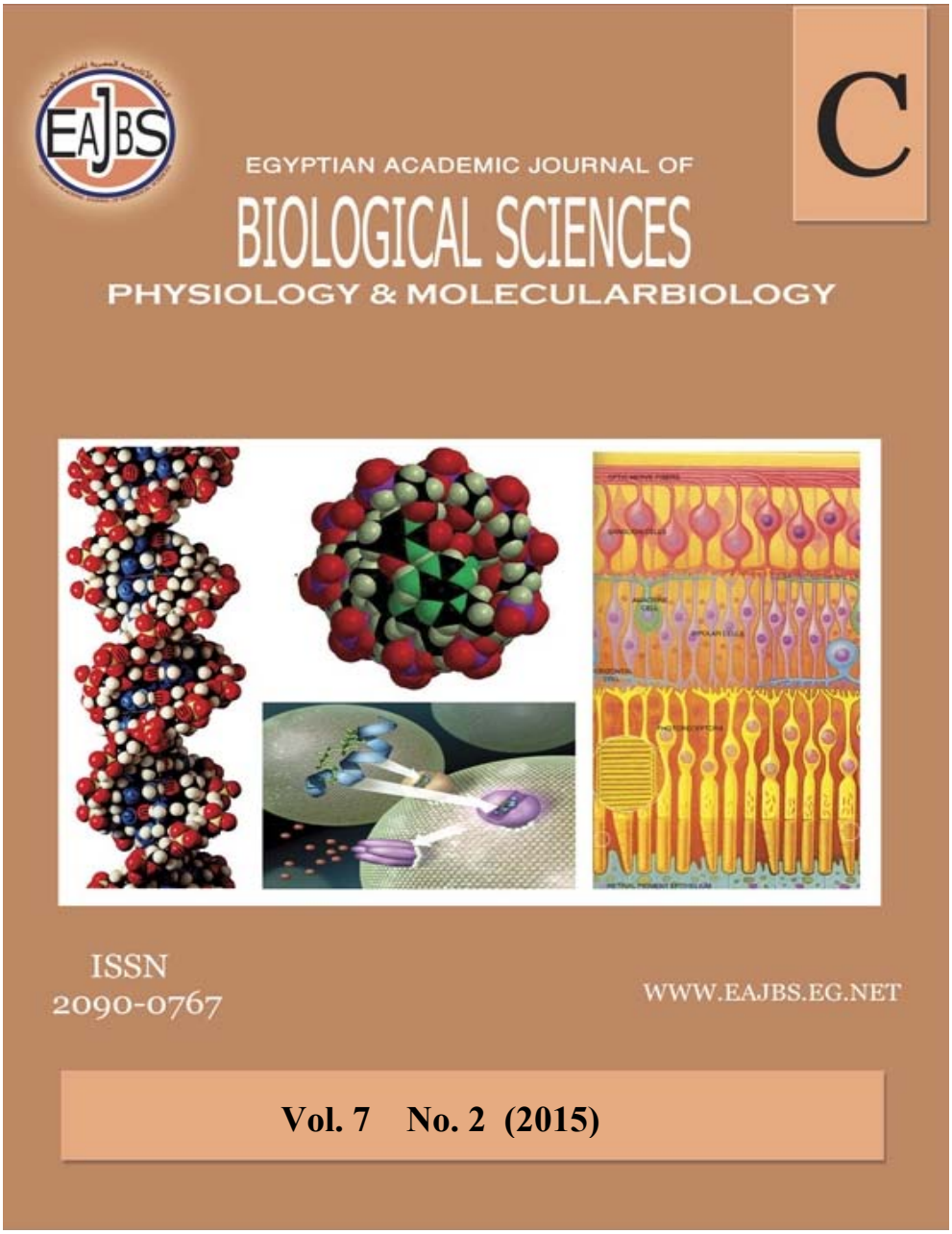

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Bioinformatic Analysis of the Beauvericin Gene from *Beauveria bassiana* and Insecticidal Effect on *Spodoptera littoralis* (Boisd)

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ABSTRACT

Beauvericin, a cyclohexadepsipeptide-possessing natural product with synergistic antifungal, insecticidal, and cytotoxic activitie. Total DNA was extracted from *B. bassiana* Egyptian isolate. The integrity of total DNA was estimated by ethidium bromide re, taq DNA polymerase and (Forward and Reverse) primers directly which designed to amplify the beauvericin gene. The nucleotide sequence of the PCR-amplified fragment for the beauvericin gene of *B. bassiana* EG-isolate was done to determine the relationship with other *B. bassiana* isolates registered in Gen Bank, and was aligned by using DNAMAN program (Wisconsin, Madison, USA) with another three *B. bassiana* isolates. The predict numbers of amino acids were produced from translation of beauvericin gene nucleotide sequence were 211 amino acids. A phylogenetic tree of beauvericin from *B. bassiana* Eg. isolate revealed 100% a high degree of similarity to beauvericin of *B. bassiana* non ribosomal cyclo depsipeptide synthetase, (Accession no. AC130655), 94.3% and 94.8% to beauvericin of *B. bassiana* biosynthetic protein (Accession no. ADO 60131) and beauvericin of *B. bassiana* biosynthetic protein, (Accession no. AFJ44691) respectively. While, the insecticidal effect of toxins crude extraction was studied in this wake . Toxins crude extraction due to isolate of *B. bassiana* investigated against 3rd inster larvae of *S. littoralis*. After 4 days, the percentage of mortality were 51.00%, 57.50%, 79.00% and 96.50% in the concentrations 25, 50, 75 and 100%, respectively.

INTRODUCTION

Beauvericin is a famous mycotoxin produced by many fungi, such as *B. bassiana* and *Fusarium* spp. (Logrieco, *et al*. 1998). Beauvericin was first isolated from *B. bassiana*, which is a common and commercial entomopathogenic mycoinsecticide (Hamill, *et al*. 1969). In general, the fungus then multiplies within the insect body and kills it.

Death occurs due to toxin production by the fungus and/or multiplication to inhabit the entire insect body (Goettel *et al.,* 2010). Entomopathogenic fungi are prolific producers of bioactive secondary metabolites (Isaka *et al.,* 2003; Molnar *et al.,* 2010), which are predicted to play key roles as virulence factors for fungi, infecting arthropods (Rohlfs and Churchill, 2011). Metabolites produced by entomopathogenic fungi would serve one or more of the following functions: (1) toxic to the host and help to cause death; (2) aid the fungus overcome host defense; (3) suppress competition from other pathogens and saprophytes on the insect cadaver; (4) provide a defense outside the host against other organisms (Charnley, 2003). The beauvericin synthetase purified from *B. bassiana* (Peeters *et al.,* 1988). Beauvericin belongs to the cyclic non ribosomal hexadepsi peptide family of natural products, originally isolated from entomopathogenic fungi, including *B. bassiana* (Hamill, *et al*. 1969). *B. bassiana* produces several toxic compounds (Strasser *et al*., 2000; Vey *et al*., 2001). A majority of these insecticidal molecules are low molecular weight biologically active secondary metabolites (Zimmermann, 2007). Beauvericin, bassianin, bassianolide, beauverolides, beauveriolides, tenellin, oosporein (Strasser *et al.,* 2000; Vey *et al.,* 2001), oxalic acid (Roberts, 1981) bassiacridin (Quesada- Moraga and Vey, 2004) are some of these important metabolites. These compounds also contributed in *B. bassiana* pathogenicity as they act as immune suppressors and host specific toxins (Von Dohren, 2004).

MATERIALS AND METHODS

Cultivation of *Beauveria bassiana* isolate:

The entomopathogenic fungi; *B. bassiana* (AUMC 9896) Egyptian isolate was isolatedin Bio-insectcide Production Unit, Plant Protection Research Institute and was identified in Mycological Center, Faculty of Science, Assiut University. Sahar and Moharram (2014). The isolate was cultured on Czapek Dox Brothmedium for 5 days at 25^{o}C and aseptically filtered through sterile filter paper.

DNA Extraction

DNAwas purified from *B. bassiana* according to CTAB (hexadecy ltrimethylammonium bromide) extraction method described by Doyle & Doyle (1987).

PCR Amplification:

1- Primer design:

A simple way for the primer design was used based on the alignment of *B. bassiana* beavericin gene sequences from National Center for Biotechnology Information (NCBI) (Homepage: www. Ncbi.nim.nih.gov).

The forward primer: CCGTTTCCAGTGTCTGACGA and the reverse one :AAAAGCCCGAGGCATCTTGA

2- Amplification of beauvericin gene from *B. bassiana* isolate:

PCR amplification was performed in a total volume 50µl which contained 5µl of 10x reaction buffer (600mM tris HCL pH 8.3, 250mM KCL, 1% triton X 100, 100mM B-mercaptoethanol, 2mM $MgCl_2$), 5 µl of 1mM dNTps, 2.5 µl Taq DNA polymerase, 1 µl of each primer and 1 µl of template DNA. The amplification was carried out using UNO-The rmoblock system from Biometra. Hard denaturation of the DNA was performed at 94^{o}C for 1min followed by 35 cycles of amplification with denaturation at 94^{o}C for 30sec, annealing at 58^{o}C for 30sec and extension at 72^{o}C for 1min. A single tailing cycle of long extension at 72^{o}C for 5min was carried out in order to ensure flush ends on the DNA molecules.

The PCR product of the beauvericin gene was determined by electrophorasis onto 1% agarose gel containing ethidium bromide (20µg/ml) in 1x TAE buffer to examine the actual size of the PCR product. Agarose gel electropholrasis was performed in DNA mini electropholrasis sub-cell. 8µl of PCR product and 8µl of standard DNA marker (100 bp ladders) was mixed with 2µl of 6x gel loading buffer. The PCR product was visualized on a UV transilluminator (wave length = 254nm) and photographed by camera.

3- Sequencing of beauvericin gene from *B. bassiana* isolate:

PCR product (DNA) was purified from agarose gel with Qiaquick PCR purification Kit (Qiagen) and partial nucleotide sequencing of beauvericin gene of *B. bassiana* was carried out by Applied Biosystems 3100 genetic analyzer (Applied Biosystems). The sequence data, multiple alignment and phylogentic relationship were translated and analyzed by DNA MAN program (Wisconsin, Madison, USA).

Insecticidal effect of crude toxins from *B. bassiana* isolate on *Spodoptera littoralis*

1-Production of crude toxins from *B. bassiana* isolate *in vitro*

A slant culture of *B. bassiana* grown on LcA medium (glycerol 0.1%, KH_2PO_4 0.08%, K_2HPO_4 0.02%, $MgSO_4.7H_2O$ 0.02%, KCl 0.02%, $NaNO_3$ 0.02% yeast extract 0.02% and agar 1.5%, pH 6.0) was used to inoculate a 250-ml Erlenmeyer flask containing 50 ml of the seed medium (glucose 2.0%, yeast extrac 0.2% $MgSO_4.7H_2O$ 0.05%, polypepton 0.5%, KH_2PO_4 0.1% and agar 0.1%, pH 6.0). The flask was shaken on a rotary shaker at 27 °C for 3 days. The seed culture (25ml) was transferred into a 1000-ml Erlenmeyer flask containing 500 ml of the production medium (glycerol 3.0%, oat meal 2.0%, dry yeast 1.0%, KH_2SO_4 1.0%, Na_2HPO_4 1.0% and $MgCl_2.6H_2O$ 0.5%). The flask was shaken on a rotary shaker 200rpm at 27 °C for 6 days**.** (Fukuda *et al*., 2004). the cultures were filtered through three layers of filter papers and then through what man no.1 filter paper. The culture filtrate production of crude toxins *in vitro*.

2-Rearing of insect:

The Cotton Leaf worm, *S. littoralis* larval were obtained from the department of cotton leaf worm, Plant Protection Research Institute.

Bioassay

The crude of toxin, was extracted from isolate of *B. bassiana* and four concentrations prepared (100%, 75%, 50% and 25%). 500 µl. From each dilution were added to 5 gm of diet were put into plastic container (15 cm diameter) were contains 20 larvae and were covered with muslin cloth for aeration. The larvae were left to feed on treated diet for 48 h., then mortality percentages were recorded, the survival larvae were transferred to feed on untreated diet. Mortality percentages were corrected (Ortiz-Urquiza *et al*., 2010), the lethal concentration of 50 and 90 % from treated was calculated by Probit analysis (Finney, 1971).

RESULTS

Total DNA was extracted from *B. bassiana* EG-isolate. The integrity of total DNA was estimated by ethidium bromide agarose gel electrophorasis assay, and the purity of the total DNA obtained which was 1.8 measured by spectrophotometer A260/280 absorbance ratio for indicating high yield and purity of the extracted DNA. Following DNA extraction the beauvericin gene in the isolate genome was amplified using PCR technique. 1µl of DNA was mixed with PCR reaction mixture, taq DNA polymerase and (Forward and Reverse) primers directly which designed to amplify the beauvericin gene.

Extraction and amplification of DNA-beauvericin gene:

Total DNA was extracted from *B. bassiana* Egyptian isolate. The integrity of total DNA was estimated by ethidium bromide re, taq DNA polymerase and (Forward and Reverse) primers directly which designed to amplify the beauvericin gene.

Electrophorasis analysis of PCR product:-

The size of the PCR product amplified from *B. bassiana* was estimated after running in 1% agarose gel electrophorasis by comparing its electrophorasis mobility with those of standard DNA marker as shown in (Fig. 1).

Nucleotide Sequence analysis:-

The nucleotide sequence of the PCR-amplified fragment for the beauvericin gene of *B. bassiana* EG-isolate was done to determine the relationship with other *B. bassiana* isolates registered in GenBank (Table 1). The sequencing assembly was done by analyzed the sequence results generated by the forward and reverse sequencing primers with the software program sequencing analysis. Nucleotides were found to be 636bp from beauvericin genome sequence (Fig. 2).

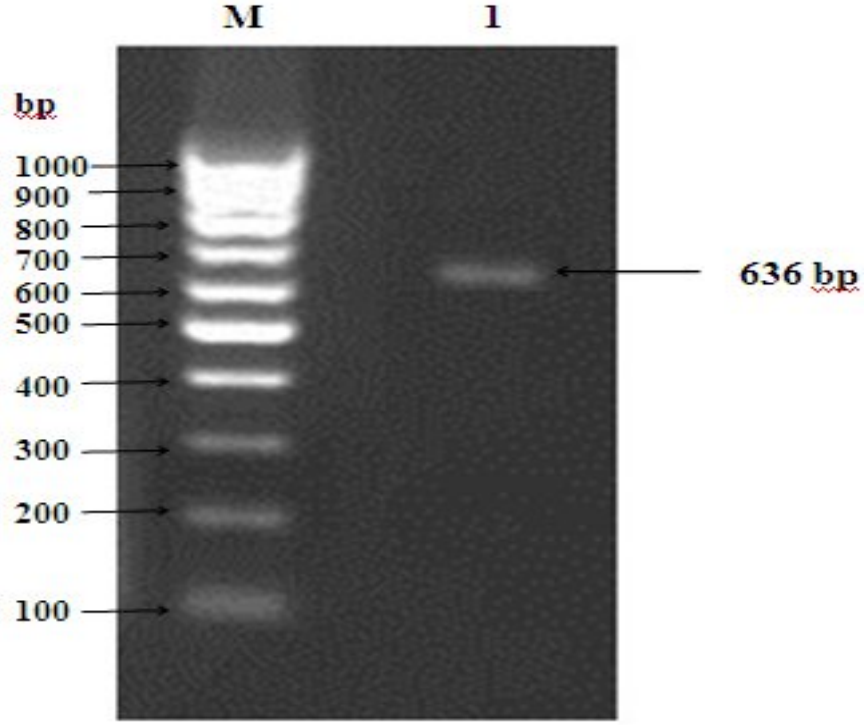

Fig. 1: 1% agarose gel electrophorasis showing PCR product amplified from total DNA extracted *B. bassiana* isolate (Lane 1), M: DNA molecular weight marker (100bp ladder).

Analysis of molecular data by bioinformatics:-

The nucleotide sequence of the beauvericin gene from *B. bassiana* isolate was aligned by using DNAMAN program (Wisconsin, Madison, USA) with another three *B. bassiana* isolates Fig. 3 which are: *B. bassiana* strain ATCC 7159 beauvericin biosynthetic gene locus, complete sequence, the USA (Accession no. EU886196) reported by Xu,Y., *et al.* (2008), *B. bassiana* isolate Bb0062 beauvericin biosynthetic protein (BVRC) gene, complete cds, China(Accession no. JQ617289) Zhou, Y., *et al.* (2012) and *B. bassiana* clone BbBVRC beauvericin biosynthetic protein gene, partial cds, China(Accession no. HQ141932) Zhou, Y., *et al.* (2010). (Table 1). The

nucleotide sequence similarity of *B. bassiana* EG-isolate with three *B. bassiana* isolates was shown in (Fig. 4). The phylogenetic tree of *B. bassiana* isolate revealed 100% a high degree of similarity to complete sequence of *B. bassiana* beauvericin biosynthetic gene, The USA (Accession no. EU886196) followed by 96.9% to *B. bassiana* isolate Bb0062 beauvericin biosynthetic protein (BVRC) gene, China (Accession no. JQ617289) and 96.5% similarity to *B. bassiana* clone BbBVRC beauvericin biosynthetic protein gene China (Accession no. HQ141932).

```
  1 CGTTTCCAGT GTCTGACGAG ACAGTTGAGC ATTTGAATGG TCTATATGAGGAAATCAACC
 61 GCCGTTTTGG CTTGGACAGG GATGCCATTG AGACTATCCT CCCATGTACA CCCTTCCAGT
121 ATGATGTGCT TGATTGCGCT GCCAATGATG CAAGACACGC CGTCGGTCAT GCCATGTACG
181 AAATATCGCA ACATGTTCAT GTCCAACGCT TCATCGCTGC TTGGAGAGAG ACTGTGCGGC
241 GCACTCCAGC CTTGCGCGCC TGCACCTTTA CATCAACGAC CGGGGAGTCG TTTCAGCTGG
301 TACTGAGAGA GAGCTTTGTG CTTTCGCGCA TATACTGGTC TTCTTCTTCT AGCTTACAGG
361 CAGCTGTTTT GAAGGATGAG ACGACGGCGG CCATTGCTGG GCCGCGTTGC AATCGACTTG
421 TCCTTCTTGA AGACCCAGAT ACAAGGAAAC AACTGCTGAT TTGGGTATTT CATCTTGCAC
481 TCGTGGACAG CACCGTTCAG GAACCCATTC TCCGGCGGGT TCTGGCGGCG TACAAGAGTG
541 AAGACGACCA GCTAGACAGC CTTCCGCTCA CACCAGACTC TTCTGGAGGT TCCGACTCGG
601 ACTCTCCCAG CACGCTCAAG ATGCCTCGGG CTTTTG
```

Fig. 2: The nucleotide sequence of DNA (636bp) from beauvericin gene of *B. bassiana* isolate.

Table 1: Referencesfor beauvericin gene from different three isolates of *B.bassiana*

Accession no.	**Authors**	**References**	Country
EU886196	Xu,Y., Orozco, R., Wijeratne, E. M., Gunatilaka, A. A., Stock, S. P. and Molnar, I.	Xu,Y., *et al* (2008)	USA
JQ617289	Zhou,Y., Zhang,Y., Luo, Z. and Pei,Y.	Zhou,Y., *et al* (2012)	China
HQ141932	Zhou, Y., Zhang,Y., Jin, K., Luo, Z. and Pei,Y.	Zhou,Y., *et al* (2010)	China

```
Beauveria_bassiana_Egyptian_isolate   CGTTTCCAGT GTCTGACGAG ACAGTTGAGC ATTTGAATGG TCTATATGAG GAAATCAACC GCCGTTTTGG CTTGGACAGG  [ 80]
EU886196                              .......... .......... .......... .......... .......... .......... .......... ..........  [ 80]
HQ141932                              .......... .......... .......... ..C....... .T........ .......... .......... .G........  [ 80]
JQ617289                              .......... .......... .......... ..C....... .......... .......... .......... .G........  [ 80]

Beauveria_bassiana_Egyptian_isolate   GATGCCATTG AGACTATCCT CCCATGTACA CCCTTCCAGT ATGATGTGCT TGATTGCGCT GCCAATGATG CAAGACACGC  [160]
EU886196                              .......... .......... .......... .......... .......... .......... .......... ..........  [160]
HQ141932                              ........C. .......... .......... .......... .C........ .......... .......... ..........  [160]
JQ617289                              ........C. .......... .......... .......... .C........ .......... .......... ..........  [160]

Beauveria_bassiana_Egyptian_isolate   CGTCGGTCAT GCCATGTACG AAATATCGCA ACATGTTCAT GTCCAACGCT TCATCGCTGC TTGGAGAGAG ACTGTGCGGC  [240]
EU886196                              .......... .......... .......... .......... .......... .......... .......... ..........  [240]
HQ141932                              .......... ....C..... .......... .......... .......... .A........ .......... ..........  [240]
JQ617289                              .......... ....C..... .......... .......... .......... .A........ .......... ..........  [240]

Beauveria_bassiana_Egyptian_isolate   GCACTCCAGC CTTGCGCGCC TGCACCTTTA CATCAACGAC CGGGGAGTCG TTTCAGCTGG TACTGAGAGA GAGCTTTGTG  [320]
EU886196                              .......... .......... .......... .......... .......... .......... .......... ..........  [320]
HQ141932                              .......C.. .......... .......... .......... T......... ........A. .......... ..........  [320]
JQ617289                              .......C.. .......... .......... .......... T......... ........A. .......... ..........  [320]

Beauveria_bassiana_Egyptian_isolate   CTTTCGCGCA TATACTGGTC TTCTTCTTCT AGCTTACAGG CAGCTGTTTT GAAGGATGAG ACGACGGCGG CCATTGCTGG  [400]
EU886196                              .......... .......... .......... .......... .......... .......... .......... ..........  [400]
HQ141932                              .......... .......... .......... .....GAC.A .......... .......... ...G...... .TG..T....  [400]
JQ617289                              .......... .......... .......... .....GAC.A .......... .......... ...G...... .TG..T....  [400]

Beauveria_bassiana_Egyptian_isolate   GCCGCGTTGC AATCGACTTG TCCTTCTTGA AGACCCAGAT ACAAGGAAAC AACTGCTGAT TTGGGTATTT CATCTTGCAC  [480]
EU886196                              .......... .......... .......... .......... .......... .......... .......... ..........  [480]
HQ141932                              .......... .......... ..T....... .......... ....A..... .......... .......... ..........  [480]
JQ617289                              .......... .......... .......... .......... ....A..... .......... .......... ..........  [480]

Beauveria_bassiana_Egyptian_isolate   TCGTGGACAG CACCGTTCAG GAACCCATTC TCCGGCGGGT TCTGGCGGCG TACAAGAGTG AAGACGACCA GCTAGACAGC  [560]
EU886196                              .......... .......... .......... .......... .......... .......... .......... ..........  [560]
HQ141932                              .......... ......C... .......... .......... .......... .......... .......... ..........  [560]
JQ617289                              .......... ......C... .......... .......... .......... .......... .......... ..........  [560]

Beauveria_bassiana_Egyptian_isolate   CTTCCGCTCA CACCAGACTC TTCTGGAGGT TCCGACTCGG ACTCTCCCAG CACGCT---C AAGATGCCTC GGGCTTTTG [639]
EU886196                              .......... .......... .......... .......... .......... .......--- .......... ..........  [639]
HQ141932                              ........T. .......... .......... .......... .......... ......ACT. .......... ..........  [639]
JQ617289                              ........T. .......... .......... .......... .......... ......ACT. .......... ..........  [639]
```

Fig. 3: Multiple sequence alignment of the nucleotide sequence of *beauvericin* gene from *B. bassiana* Eg. Isolate and the other isolates of *B. bassian* available in Gen Bank.

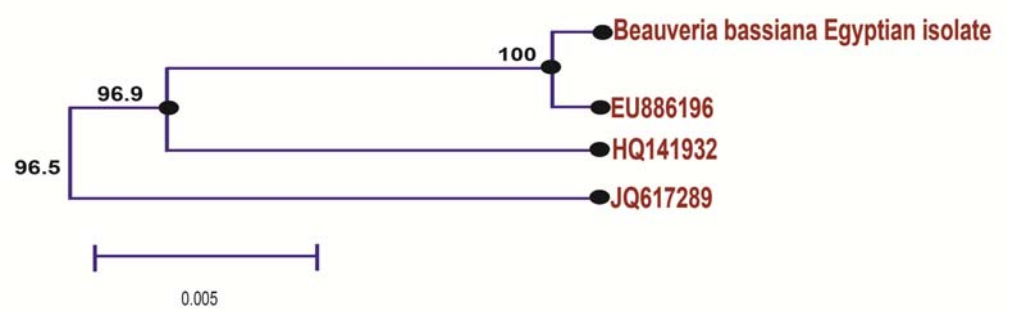

Fig. 4: Phylogenetic tree of *beauvericin gene;* the dendrogram displaying the percentage of sequence homology between *beauvericin gene* from *B. bassiana E*g. isolate and the other three *B. bassiana* published in GenBank.

Translation of nucleotide sequence of beauvericin gene from *B. bassiana* Eg. Isolate :-

The predict numbers of amino acids were produced from translation of beauvericin gene nucleotide sequence were 211 amino acids (Figs. 5&6).

```
1 CGTTTCCAGTGTCTGACGAGACAGTTGAGCATTTGAATGGTCTATATGAGGAAATCAACC
FPVSDETVEH LNGLYEEIN
61 GCCGTTTTGGCTTGGACAGGGATGCCATTGAGACTATCCTCCCATGTACACCCTTCCAGT
    R  R  F  G  L  D  R  D  A  I  E  T  I  L  P  C  T P F Q
121 ATGATGTGCTTGATTGCGCTGCCAATGATGCAAGACACGCCGTCGGTCATGCCATGTACG
YDVLDCAANDA RHAVGHAMY
181 AAATATCGCAACATGTTCATGTCCAACGCTTCATCGCTGCTTGGAGAGAGACTGTGCGGC
EISQHVHVQRF IAAWRETVR
241 GCACTCCAGCCTTGCGCGCCTGCACCTTTACATCAACGACCGGGGAGTCGTTTCAGCTGG
RTPALRACTFT STTGESFQL
301 TACTGAGAGAGAGCTTTGTGCTTTCGCGCATATACTGGTCTTCTTCTTCTAGCTTACAGG
VLRESFVLSRI YWSSSSSLQ
361 CAGCTGTTTTGAAGGATGAGACGACGGCGGCCATTGCTGGGCCGCGTTGCAATCGACTTG
AAVLKDETTAA IAGPRCNRL
421 TCCTTCTTGAAGACCCAGATACAAGGAAACAACTGCTGATTTGGGTATTTCATCTTGCAC
VLLEDPDTRKQ LLIWVFHLA
481 TCGTGGACAGCACCGTTCAGGAACCCATTCTCCGGCGGGTTCTGGCGGCGTACAAGAGTG
LVDSTVQEPIL RRVLAAYKS
541 AAGACGACCAGCTAGACAGCCTTCCGCTCACACCAGACTCTTCTGGAGGTTCCGACTCGG
EDDQLDSLPLTPDSSGGSDS
601 ACTCTCCCAGCACGCTCAAGATGCCTCGGGCTTTTG
     D  SPSTLKMPRA  F
```

Fig. 5: Translation of nucleotide sequence of beauvericin gene from *B. bassiana* isolate produced 211 amino acids with MW=23.373KDa

```
  1 FPVSDETVEH LNGLYEEINR
 21 RFGLDRDAIETILPCTPFQY
 41 DVLDCAANDA RHAVGHAMYE
 61 ISQHVHVQRF IAAWRETVRR
 81 TPALRACTFT STTGESFQLV
101 LRESFVLSRI YWSSSSSLQA
121 AVLKDETTAA IAGPRCNRLV
141 LLEDPDTRKQ LLIWVFHLAL
161 VDSTVQEPIL RRVLAAYKSE
181 DDQLDSLPLT PDSSGGSDSD
201 SPSTLKMPRA F
```

Fig. 6: 211 amino acids sequence of beauvericin gene from *B. bassiana* isolate.

The amino acids composition of beauvericin gene sequence for *B. bassiana* Eg. Isolate was aligned by using DNAMAN program (Wisconsin, Madison, USA) with three *B. bassiana* isolates Fig. 7, which are: Beauvericin of *B. bassiana* non ribosomal cyclodepsipeptide synthetase, (Accession no. AC130655), USA, Xu,Y., *et al.* (2008), beauvericin of *B. bassiana* biosynthetic protein, (Accession no. ADO60131), China, Zhou, Y., *et al.* (2010) and beauvericin of *B. bassiana* biosynthetic protein, (Accession no. AFJ44691), China, Zhou,Y., *et al.* (2012).

The amino acids composition of beauvericin gene sequence similarity of *B. bassiana* Eg. isolate with three published isolates of *B. bassiana* was shown in Fig. (8). A phylogenetic tree of beauvericin from *B. bassiana* Eg. isolate revealed 100% a high degree of similarity to beauvericin of *B. bassiana* nonribosomal cyclodepsipeptide synthetase, (Accession no. AC130655), 94.3% and 94.8% to beauvericin of *B. bassiana* biosynthetic protein, (Accession no. ADO60131) and beauvericin of *B. bassiana* biosynthetic protein, (Accession no. AFJ44691) respectively, Fig. 8.

```
Beauveria_bassiana_Egyptian_isolate   FPVSDETVEH LNGLYEEINR RFGLDRDAIE TILPCTPFQY DVLDCAANDA RHAVGHAMYE ISQHVHVQRF IAAWRETVRR  [ 80]
ACI30655                              .......... .......... .......... .......... .......... .......... .......... ..........  [ 80]
ADO60131                              .......... .......... ...V...... .......... .......... .......T.. .........L ..........  [ 80]
AFJ44691                              .......... .......... ...V...... .......... .......... .......T.. .........L ..........  [ 80]

Beauveria_bassiana_Egyptian_isolate   TPALRACTFT STTGESFQLV LRESFVLSRI YWSSSSSLQA AVLKDETTAA IAGPRCNRLV LLEDPDTRKQ LLIWVFHLAL  [160]
ACI30655                              .......... .......... .......... .......... .......... .......... .......... ..........  [160]
ADO60131                              .......... .......... .......... ........TT .......A.. VS........ F......K.. ..........  [160]
AFJ44691                              .......... .......... .......... ........TT .......A.. VS........ .......K.. ..........  [160]

Beauveria_bassiana_Egyptian_isolate   VDSTVQEPIL RRVLAAYKSE DDQLDSLPLT PDSSGGSDSD SPST-LKMPR AF [212]
ACI30655                              .......... .......... .......... .......... ....-..... .. [212]
ADO60131                              .......... .......... .......... .......... ....L..... .. [212]
AFJ44691                              .......... .......... .......... .......... ....L..... .. [212]
```

Fig. 7: Multiple amino acid sequence alignment of beauvericin gene from *B. bassiana* Eg. Isolate with the corresponding amino acid sequence of beauvericin gene from three *B. bassiana* isolates available in Gen Bank.

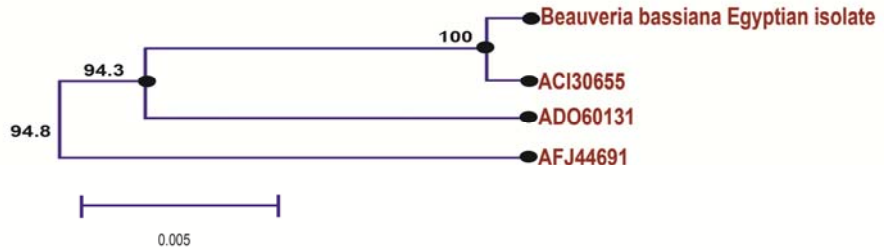

Fig. 8: Phylogenetic tree of the beauvericin gene from *B. bassiana* Eg. isolate based on the amino acid sequence; the denderogram displaying the percentage of amino acid sequence homology between the beauvericin gene of *B. Bassiana* Eg. Isolate and the other three isolates of *B. bassiana* published in Gen Bank.

Insecticidal effect of toxins crude extraction

Toxins crude extraction due to isolate of *B. bassiana* investigated against 3rdinster larvae of *S. littoralis*as shown in Table (2). After 1 day, the crude of *B. bassiana* gave 5.00%, 10.50%, 17.00% and 30.00% mortality in the concentrations 25, 50, 75 and 100%, respectively. The second day the percentage of mortality were 15.50%, 30%,55.50% and 60.50%in the concentrations 25, 50, 75 and 100%, respectively. After 4 days, the percentage of mortality were 51.00%, 57.50%, 79.00% and 96.50% in the concentrations 25, 50, 75 and 100%, respectively.

Table 2: Corrected a cumulative mortality percentages of 3rd instars larvae of *S. littoralis* after feeding on synthetic diet treated with metabolic toxins crude

Conc. (%)	*Cumulative mortality % at indicated days after treatment*			
	1	**2**	**3**	**4**
25	5.00	15.50	36.00	51.00
50	10.50	30.00	56.00	57.50
75	17.00	55.50	62.50	79.00
100	30.00	60.50	75.00	96.50

DISCUSSION

Goettel *et al*., 2010 reported that entomopathogenic fungus, *B. bassiana* is a broad host range entomopathogen that plays an importantrole in the control of insect populations in nature. This fungus is the most widely used fungal species available commercially. It is generally found on infected insects both in temperate and tropical areas throughout the world (Zimmermann, 2007). Duringits pathogenic phase, the developing hyphae directly penetrate the insect integument by producing extracellular enzymes (Fan *et al*., 2007) and *B. bassiana* produces several toxic compounds (Strasser *et al*., 2000; Vey *et al*., 2001). A majority of these insecticidal molecules are low molecular weight biologically active secondary metabolites (Zimmermann, 2007). Beauvericin, bassianin, bassianolide, beauverolides, beauveriolides, tenellin, oosporein and oxalic acid. These compounds also contributed in *B. bassiana* pathogen city as they act as immune suppressors and host specific toxins (Von Dohren, 2004). Among them, Beauvericin is the most important compound which was isolated first from *B. bassiana*. Not all isolates of *B. bassiana* produce beauvericin (Frappier *et al.,* 1975; Zimmermann, 2007), but other species produce this compound like *Fusarium* Spp. (Hamill, *et al* 1969 and Logrieco, *et al.* 1998). Beauvericin carries insecticidal properties.

Molecular methods based on PCR and DNA sequences of PCR products can greatly reduce the amount of time needed for identifying and characterization of any isolate. Polymerase Chain Reaction (PCR) technique is reported to be ingenious technique in molecular biology that allow rapid and specific amplification of DNA present in very small amounts in complex mixtures of nucleic acids, so it is a powerful technique developed for detection any gene.

Viaud *et al*., 1996 determined to talgenome size of *B. bassiana* by PCR amplification which considered highly sensitive process give desirable results. Concentration of DNA and particularly annealing temperature of primers has high importance in successful PCR amplification. For this, temperature gradient of annealing temperature from 56°C to 65°C was used in thermal cycler

and 58°C was showed to be the optimum temperature, Wang *et al*., (2003) used this temperature as optimum temperature for amplifying the pr1 gene, our results are agree with them and disagree with Viaud *et al*. 1996 who used the 65°C as annealing temperature.

Few authors described and characterized beauvericin gene from *B. bassiana* like Peeters., *et al*. (1988) and Xu., *et al*. (2008). Inaddition to *B. bassiana* Zhang Tao, *et al.* (2013) made identification and sequence analysis of beauvericin gene from *F. proliferatum*.

Beauvericin was confirmed as the active compound from *B. bassiana* against *Artimiasalina*, which was considered a model organism to study insecticidal activity. Subsequently, the insecticidal effect of beauvericin on a microgram level was investigated on *Calliphora erythrocephala*, *Aedesaegypti*, *Lygus* spp., *Spodoptera frugiperda* and *Schizaphis graminum* (Grove and Pople, 1980; Jestoi, 2008 . Leland *et al.*, 2005). Wang and Xu(2012) found that beauvericin was a strong insecticidal activity against a broad spectrum of insect pests. the insecticidal mechanism of beauvericin is still worth investigated. There are few reports about the insecticidal mechanism of beauvericin. Despite similarities between the chemical structures of beauvericin and other mycotoxins, beauvericin is more effective (Grove and Pople, 1980) and may have a unique mechanism of action. The discovery of the active mechanism of beauvericin against insects will be helpful to find new commercial insecticidal agents, reduce the threat of insecticidal agents to human cells.

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ARABIC SUMMERY

تحليل المعلومات الحيوية الخاصة بجين البفريسين المستخلص من فطر البيوفريا بسيانا ودراسة تأثيرة المميت علي حشرة دودة ورق القطن الكبري

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تم تحديد الجين الخاص بافراز توكسين البفريسين من عزلة مصرية لفطر البيوفريا باسيانا وهذا التوكسين ذو تأثير سام علي عديد من الحشرات ويفرز طبيعيا داخل الحشرة عندما تخترق بواسطة الفطر ويكون احد مسببات الموت للحشرة . وتم تحديد (البريمر) الخاص بالجين وبمقارنة تتابع النيكلوتيدات الخاصة بالجين وجد تطابقها مع نيكليوتيدات ثلاث عزلات فطرية لنفس الفطر البيوفريا باسيانا بواسطة بنك الجينات وكـذلك تركيب الاحماض الامينية للجين وبدراسة التأثير المميت للتوكسين عند انتاجة في بيئة الفطر وجد انه يعطي نسبة موت قد تصل الي 96,5 % علي يرقات دودة ورق القطن بعد مرور 4 ايام علي معاملتها بالبيئة المحتوية علي التوكسين في اعلي تركيز له .