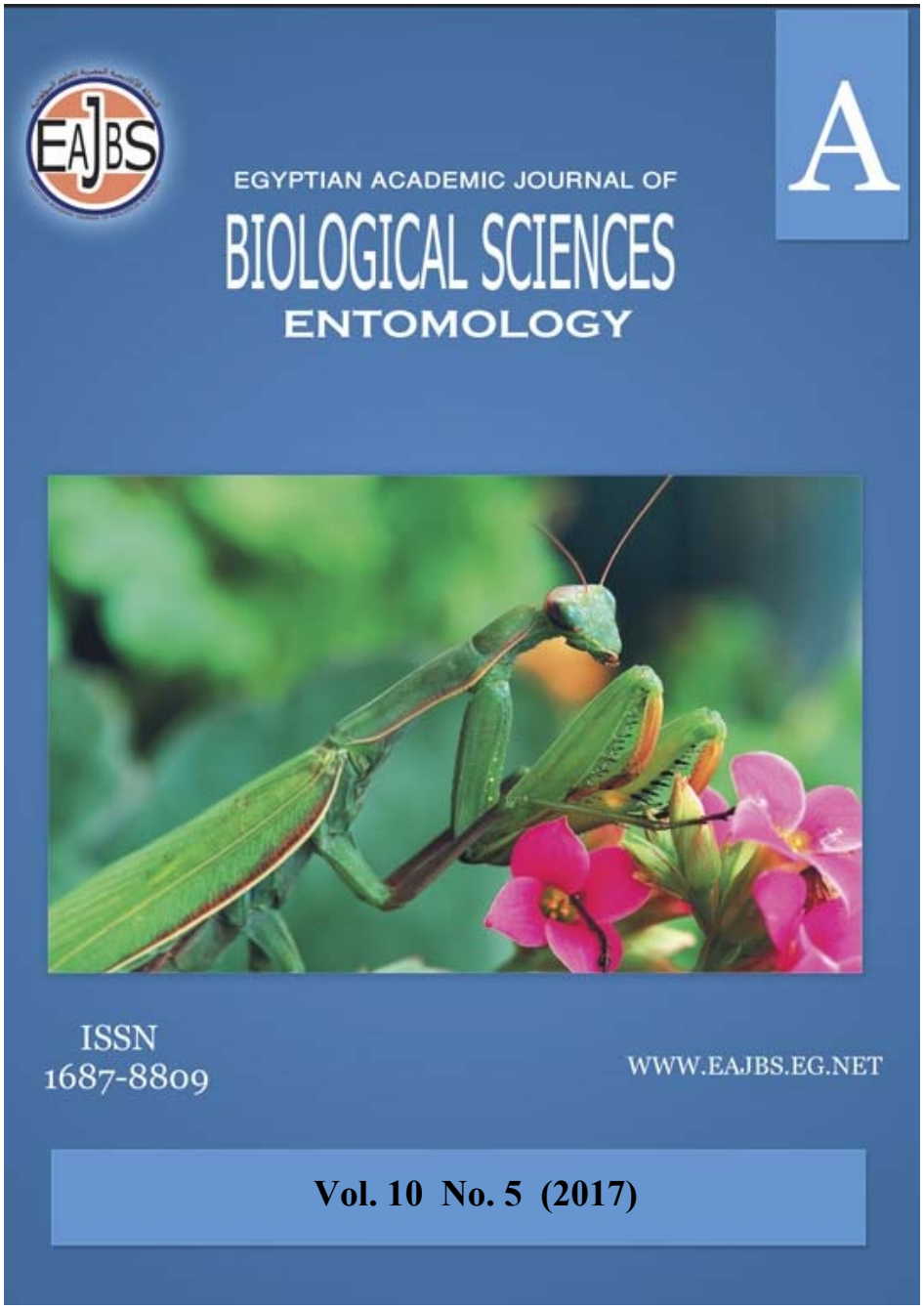

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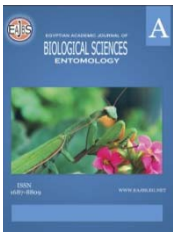

The Biochemical Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on 3rd instar Larvae of *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

The biochemical effects of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, were studied in the 3rd instar larvae of *Culex pipiens* in the laboratory. Results revealed significant reduction in the total proteins of the treated larvae compared to the control larvae. The biochemical studies using (SDS-PAGE) revealed that there was a reduction in the number of protein bands due to the treatment with either of the two fungi. The obtained results indicated that the application of entomofungi as larvicidal agents against mosquito larvae caused significant changes in the total protein profile of *Cx. pipiens* larvae suggesting that toxins secreted by these pathogens caused damage to the larval proteins which finally leads to larval death. The conclusion is that the fungal pathogens are important as natural biological control agents of many insect and other arthropods and frequently cause epizootics that significantly reduce host population. This study recommends the use of *B. bassiana* and *M. anisopliae* as biological control agents to the control of *Cx. pipiens* larvae in order to suppress this medical vector for public health.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) species are vectors responsible for the transmission of several infectious diseases with medical and veterinary importance including filariasis, malaria, and arboviruses (Goddard 2008; Mullen & Durden 2009; Medlock *et al*. 2012). Risk for human infection considerably enlarged during the last decades due to climate changes and increasing global trade (Reiter 2001; Medlock *et al*. 2012; Boukraa *et al*. 2013). *Culex pipiens* is one of the most annoying vectors of pathogens for humans. The best counteractive action of mosquito borne diseases is accomplished by decreasing the mosquito population in any of the different life cycle stages, for example, using larvicidal substances. Currently, some problems are caused by the multiple usages of chemical insecticides and reported with respect to the persistence and increase of non-biodegradable chemicals in the environment, the biological enlargement through the food chain, the toxic effect to human health and to non-target organisms, and the increase of insecticide resistance (Rawani *et al.* 2009).

Integrated pest management is now encouraged due to harmful side effects of

the chemical insecticides classically used for mosquito control and insect resistance development (Nauen 2007; Rattner 2009; Rivero *et al*. 2010).

Beauveria bassiana and *Metarhizium anisopliae* fungus strain is highly specific biopesticide to Mosquitoes and is considered safe to non-target invertebrates.

B. bassiana and *M. anisopliae* have been extensively studied due to their simple life-cycle and thereby easy production of steady aerial spores which are the infectious propagules (Scholte *et al.* 2004; Kanzok & Jacobs-Lorena 2006; Seye *et al.* 2013). The infection process starts by contact of the spores to the host cuticle. Sometimes, conidium attaches to the cuticle or secretes mucus for adhesion during its germination and swelling (Hajek and St. Leger, 1994). Some structures and broad process are involved in the penetration of the host cuticle and the mechanism of different fungus may also vary. After the penetration through the cuticle and insect epidermis, the fungus multiplies into the body cavity of insect and also more fungi are adapted to aquatic environments or simply ingested by larvae. Some ingested spores may mechanically block the mouth parts while others can attach inside the digestive tract (Federici, 1981; Butt *et al.* 2013).

The aim of this study was to evaluate the biochemical changes in the 3rd instar larvae of *Cx. pipiens* after exposure to biopesticide fungi *B. bassiana* and *M. anisopliae* under laboratory conditions

MATERIALS AND METHODS

Insect culture:

Mosquitoes were maintained in insectaries under controlled laboratory conditions of temperature (27±2^{o}C), relative humidity 70-80%, and light –dark period (8-16 hrs).

Larvae were reared in white enamel pan containing 1.5 liter of de-chlorinated tap water. Larvae were provided with tetra-amine (tropical fish food) sprinkled twice daily over the water surface of breeding pans. The breeding water was slightly aerated with a small air pump for about 5 minutes every day. Developed pupa were transferred daily to plastic cup containing de-chlorinated tap water then moved into the breeding screened wooden cages (30*30*30 cm3).

Emerged adults were fed on 10% sucrose solution. Adults were fed on blood after three days to lay egg-batches which moved to the white enamel pans containing de-chlorinated tap water for hatching. When mosquito larvae developed to the 2nd instar, they were poured into clean pans and observed daily. Early third larval instars were treated by the LC_{50} for each fungus according to the method described by Gerberg (1979).

Fungi source:

Entomopathogenic fungus, *Metarhizium anisopliae* and *Beauveria bassiana* was procured from Assiut University Mycological Centre (AUMC), Faculty of Science, Assiut University, 71516 Assiut, Egypt. Potato dextrose agar medium was used for fungi culturing. Culturing technique was prepared according to American Type Culture Collection (1980).

Preparation of samples for biochemical analysis:

For biochemical analysis, insect bodies were homogenized using treatment buffer (1 g insect body / 1 ml) in a chilled glass Teflon tissue grinder for 3 min according to a modified (Laemmli, 1970) method. Homogenates were centrifuged at 14000 r.p.m for 15 min at 2^{o}C in a refrigerated centrifuge. The supernatant was separated for the biochemical determination. Five replicates were used for each determination test. One replicate was used for the poly acrylamide gel

electrophoresis.

Quantitative analysis of total protein:

Total protein reagent was determined by the method of (Bradford, 1976), using Coommasie Brilliant Blue G-250 reagent (CBB). Sample solution of 50 µl was pipetted using a micropipette into a test tube and the volume was adjusted to 0.1 ml with phosphate buffer (pH 6.6). Five ml from protein reagent were added to the test tube and were mixed well. Absorbance at 595 nm was measured by using UV-1100 spectrophotometer (Chrom Tech, Inc.) after 2 minutes against blank prepared from 0.1 ml of phosphate buffer (pH 6.6) with 5 ml of protein reagent. The weight of protein was plotted against the corresponding absorbance in the standard curve which used to determine the unknown protein concentration.

Qualitative analysis of total protein:

One-dimensional gel electrophoresis was perfored in vertical polyacrylamide gels. Total larval proteins were profiled by SDS-polyacrylamide gel according to (Laemmli, 1970). SDS-molecular standard mixture of proteins; 245 kDa: 5 kDa (Sigma-Aldrish, Germany) was used as a marker with a 4% stacking gel and a 10% separating gel, at 100 volts for (5 hrs.) at room temperature. After electrophoresis, protein bands were stained for 2 hrs. in Coomassie brilliant blue (CBB.R-250), then destained for 24 hrs. for visualization of bands.

Data analysis:

The data were recorded, tabulated and subjected to statistical analysis using a software SPSS test program version 20 for Windows. The significance of the main effects was obtained by One-way analysis of variance (ANOVA) and followed by *post-hoc* analysis using LSD-test. The significance level of different treatments was evaluated at $P < 0.05$).

RESULTS

Quantitative analysis of total protein of untreated and treated 3rd instar larvae of *Cx. pipiens*:

The effect of sub-lethal concentrations (LC_{50}) of the two entomofungi on total protein content of the 3rd instar larvae of *Cx. pipiens* were recorded in (Table 1) and graphically illustrated in (Fig.1). Total protein contents were 9.23 ± 0.15 mg/ml and 3.6 ± 0.31 mg/ml in treated samples of *Cx. pipiens* larvae with *B. bassiana* and *M. anisopliae*, respectively as compared with 12.4 ± 0.23 mg/ml in the control samples. The total protein contents were significantly decreased after treatment with LC_{50} of both fungi. The total protein contents were decreased by 26% and 71% in treated larvae with *B. bassiana* and *M. anisopliae*, respectively.

Table 1: Mean values of total protein concentrations of normal and treated *Culex pipiens* larvae with the LC_{50} of two fungi (*Beauveria bassiana* and *Metarhizium anisopliae*).

Type of fungi treatment	Total protein in mg/ml
Control	12.4 ± 0.23 a
Beauveria bassiana	9.23 ± 0.15 b
Metarhizium anisopliae	3.6 ± 0.31 c

*Data are presented as mean ± SE

*Means with different letters within column are significantly different (P<0.05) ANOVA, LSD test.

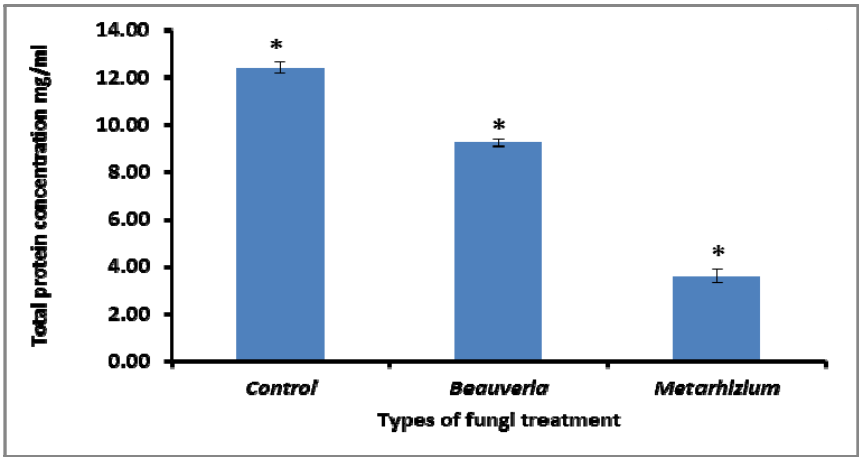

Fig 1: Changes in the concentration of total protein of normal and treated *Culex pipiens* larvae with the LC_{50} of two fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). The asterisks show significant differences at (P <0.05). Confidence bars are revealed for ± SE.

Qualitative analysis of total protein of untreated and treated 3rd instar larvae of *Cx. pipiens* using polyacrylamide gel electrophoresis:

Changes in the protein fractionation profile of untreated and treated 3rd instar larvae of *Cx. pipiens* were analyzed after 48 hrs of treatment and recorded using (SDS-PAGE). The fraction electrophoretic protein patterns were shown in (Table 2), and presented in (Fig 2 & 3).

The SDS protein patterns of larval proteins showed different numbers of protein bands according to their molecular weights (Table 2). They were separated into 24 protein bands with molecular weight ranged from 263.07 to 4.88 kDa. The SDS protein electrophoresis revealed differences between the untreated and treated samples. The number of bands of treated samples with *Beauveria bassiana* and *Metarhizium anisopliae* were 14 and 9 bands, respectively compared with 21 bands in the control samples. Bands appeared in the control samples were with molecular weights ranged from 176.79 to 4.88 kDa. The total numbers of bands in samples treated with *B. bassiana* were with molecular weights ranged from 263.07 to 7.24 kDa.

The total numbers of bands in samples treated with *M. anisopliae* were with molecular weights ranged from 75 to 6.45 kDa.

There were 6 common bands between control and treated samples appeared in r7, r9, r13, r15, r21, and r22 with molecular weight approximately 67, 58, 23, 21, 11, and 7 kDa, respectively. There were 6 characteristic bands for the control sample with molecular weight 176.79, 104.18, 50.81, 31.93, 20.79, and 4.88 kDa and percentage amount 3.79, 3.45, 4, 4.41, 3.04, and 4.01%, respectively.

There was one characteristic band for the treated sample with *B. bassiana* with molecular weight 263.07 kDa and percentage amount 4.83%.

There was one characteristic band for the treated sample with *M. anisopliae* with molecular weight 6.45 kDa and percentage amount 4.78%.

Treatment with LC_{50} of *B. bassiana* caused the disappearance of 7 bands while treatment with *M. anisopliae* caused the disappearance of 12 bands.

The obtained results indicated that the application of entomofungi as larvicidal agents against mosquito larvae caused significant changes in the total protein profile of *Cx. pipiens* larvae.

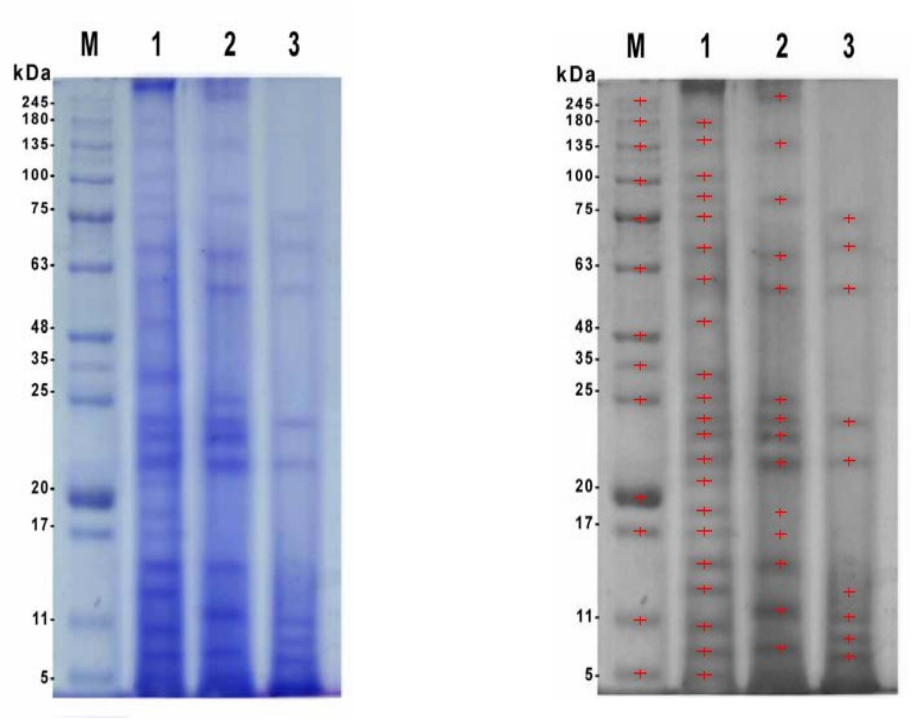

Fig 2: Electrophoretic protein pattern of SDS-PAGE of control and treated 3rd instar larvae of *Cx. pipiens* after 48 hrs. M: Marker, Lane 1: Control samples, Lane 2: Samples treated with LC_{50} *Beauveria bassiana*, Lane 3: Samples treated with LC_{50} *Metarhizium anisopliae*

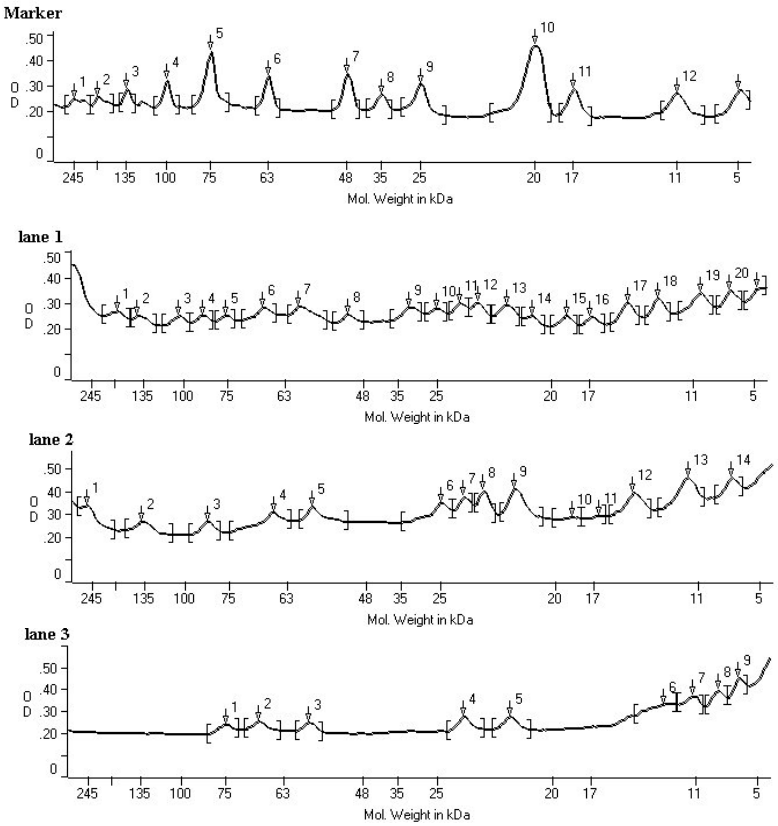

Fig 3: Denistometric scanning of SDS-electrophoresis protein patterns of control (Lane 1) and treated 3rd instar larvae of *Cx. pipiens* with LC_{50} *Beauveria bassiana* (Lane 2) and with LC_{50} *Metarhizium anisopliae* (Lane 3).

Table 2: Molecular weights of SDS-electrophoretic protein patterns for both untreated and treated samples of 3rd instar larvae of *Cx. pipiens* by *Beauveria bassiana* and *Metarhizium anisopliae* after 48 hrs.

Lanes:	Marker	Control	Samples treated with *Beauveria bassiana*	Samples treated with *Metarrhizium ansiopaliae*
Rows	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
r1	245		263.07	
r2	180	176.79		
r3	135	145.07	139.94	
r4	100	104.18		
r5		88.704	86.603	
r6	75	75.904		75
r7		67.624	65.807	67.994
r8	63			
r9		60.272	58.028	58.028
r10	48	50.811		
r11	35	31.931		
r12	25	25.385	25	
r13		23.96	23.96	23.791
r14		23.126	23.044	
r15		21.852	21.698	21.774
r16		20.795		
r17	20	18.799	18.654	
r18	17	17	16.742	
r19		14.481	14.481	
r20		12.815		12.621
r21	11	10.026	11.516	11.169
r22		6.9178	7.2462	8.3279
r23				6.4529
r24	5	4.8854		

DISCUSSION

Conventional insecticides play an important role in the overall *Culex pipiens* suppression program. However, development of *Cx. pipiens* resistance to various insecticides used indicated that great efforts should be made to find effective alternative methods of control.

Resistance to chemical insecticides is widely spread among large number of insect species (Georghiou and Mellon, 1983). In response to severe use of organophosphorus insecticides against *Cx. pipiens* mosquitoes, the resistance has increased basically upsetting control efforts (Bonning and Heming way, 1991; Abuo-El-Mahasen 2007).

Although fungal pathogens have much in common with viruses, bacteria, and other insect pathogenic microbes, they are distinctive in many ways (Ferron, 1978). Feasibly the most significant difference comes from the mode of infection; whereas most entomopathogens infect their hosts through the gut after feeding. Fungi typically penetrate the insect cuticle and considered major pathogens known to infect insects with sucking mouthparts, orders Hemiptera and Homoptera (Roberts and Humber, 1981).

In the present study total protein was quantitatively estimated in untreated 3rd instar larvae of *Cx. pipiens* and treated larvae with the sublethal concentration (LC_{50}) of *B. bassiana* and *M. anisopliae*. Also qualitative analyses of treated 3rd instar larvae were studied. Total protein was conducted in a trial to detect the mode of action of the two entomopathogenic fungi.

Proteins are essential constituents of the general animal cells and also in the maintenance of different activities. These changes may be due to certain defects in enzymes that are responsible for protein and lipid synthesis. On the other hand, it is obvious that the tested biological agents decreased the protein contents in *Culex pipiens* larvae. Because proteins are essential to chitin synthesis, the depletion of this metabolic macromolecule indicates that chitin production must be inhibited. It is well known that the proteins are the major and in essential for the insect life (for energy production, for the adult fecundity, and for fertility) which were affected clearly by this biomatter.

As indicated from the obtained results, the protein contents of the treated larvae with LC_{50} of *B. bassiana* and *M. anisopliae* were significantly decreased in terms of the optical density measurements compared to the control samples. The obtained result agreed with Broome *et al*. (1976) who found that $0.36X10^8$ spore/ml decreased the total protein content in adults of boll weevil, *Anthonomus grandis* by 41%. In agreement with our study, Sree and Joshi (2015) revealed that, inoculation of fungal pathogen *Beauveria bassiana* resulted significant reduction in protein content of haemolymph in treated silkworm larvae compared to control group. Also Elbanna *et al*., (2012) confirmed that total insect protein contents were radically declined with treatments of *M. anisopliae* fungi on locusts. Sahayaraj and Borgio (2010) found that the insecticidal activity of *B. bassiana* and *M. anisopliae* also reduced total body protein content in *Dysdercus cingulatus*.

The reduction in protein level resulted from damage of protein molecule and alteration of certain amino acid side chains due to damage of protein, which leads to alteration in its properties to the point where it can no longer serve its usual purpose (Spikes and Macknight, 1970). Broome *et al*., (1976) and Callaham *et al*., (1977) suggested that this decrease might be due to accumulative energy stress on the organism, and it may be having a great effect on adult mortality.

The changes in the protein profile of the treated *Cx. pipiens* larvae with LC_{50} of the 2 entomofungi were compared by electrophoretic runs of proteins extracted from treated and untreated larva. The electrophoretic analysis of SDS-PAGE protein pattern of the tissue proteins of control and treated samples of *Cx. pipiens*, with the sub-lethal dose of the two entomofungi showed changes in protein pattern. The total numbers of bands of control samples were 21 with molecular weights ranged from 176.79 to 4.88 kDa, while in treated samples with *B. bassiana* and *M. anisopliae* there were 14 and 9 bands, respectively. These data is in agreement with our obtained results of the total protein analyses. According to this study, SDS protein pattern suggested that, there were 6 common bands with molecular weight ranged between 67 and 7 kDa, these might be characteristics for *Cx. pipiens* samples as they were presented in both treated and control samples. Our results are in agreement with El-Sonbaty *et al*., (2016) who conveyed that analysis of haemolymph protein profile of *Spodoptera littoralis* larvae using gel electrophoresis showed fourteen differentially expressed proteins ranging from 9.6 - 116.2 KDa post infection with *M. anisopliae*. The infection distinctly affected protein profiles and can be manipulated in the proteins of molecular weight in the range of 56.9-82.6 KDs of prophenoloxidases and phenoloxidases. They concluded that *M. anisopliae* infection greatly affected cellular immune system and protein expression. So, it could result into death of insect due to disturbance in the immune system and proteins. Gabarty *et al*., (2013) reported that SDS protein analysis of the *Spodoptera littoralis* larvae revealed that the immune enzymes activity and protein concentration were significantly decreased at second, third, and fourth day of treatment with *Beauveria bassiana* and *Metarhizium anisopliae*.

It can be concluded from the obtained results that the application of entomofungi as larvicidal agents against mosquito larvae caused significant changes in the total protein profile of *Cx. pipiens* larvae suggesting that toxins secreted by these pathogens caused damage to the larval proteins which finally leads to larval death. This study recommends the use of *B. bassiana* and *M. anisopliae* as biological control agents to the control of *Cx. pipiens* larvae in order to suppress this medical vector for public health.

REFERENCES

Abuo-El-Mahasen M. (2007). Histochemical effects of abamectin and *Bacillus thuringiensis* on the mid-gut and the fat bodies of the cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae. Sci. J. Fac. Sci. Menufia Univ., 21: 71-90.

American Type Culture Collection (1980). ATCC Catalog of Strains, published by the American type culture collection, Rockville, Maryland.

Bonning, B. C.; J. Hemingway; R. Romi and G. Majori. (1991). Interaction of insecticide resistance genes in field populations of *Culex pipiens* (Diptera: Culicidae) from Italy in response to changing insecticide selection pressure. Bull. Entomol. Res. 8l:5-10.

Boukraa, S., F.N. ;Raharimalala, J.-Y. ;Zimmer, F. Schaner ;T. Bawin, E. Haubruge and F. Francis. (2013). Reintroduction of the invasive mosquito species *Aedes albopictus* in Belgium in July 2013. Parasite 20: 54.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72:248–254.

Broome, J. B., Callaham, M. F., Poe, W. E., & Heitz, J. R. (1976). Biochemical changes in the boll weevil induced by rose bengal in the absence of light. *Chemico-biological interactions*, 14(1-2), 203-206.

Butt, T.; Greenfield, B.; Greig, C.; Maffeis, T.; Taylor, J.; Piasecka, J. and Quesada-Moraga, E. (2013). *Metarhizium anisopliae* pathogenesis of mosquito larvae: a verdict of accidental death. PloS one, 8(12), e 81686.

Callaham, M. F., Broome, J. R., Poe, W. E., & Heitz, J. R. (1977). Time dependence of light-independent biochemical changes in the boll weevil, Anthonomous grandis, caused by dietary rose bengal. *Environmental Entomology*, 6(5), 669-673.

Elbanna, S. M.; Elhadidy, N. M. ; Semida, F. M., and Abdel Rasool, T. (2012). Physiological and biochemical effect of entomopathogenic fungus *Metarhizium anisopliae* on the 5th instar of *Schistocerca gregaria* (Orthoptera: Acrididae). J. Res. Environ. Sci. Toxicol, 1(1), 7- 18.

El-Sonbaty, S. M.; Gabarty, A. and Ibrahim, A. A. (2016). .Hematological and Protein Response of *Spodoptera littoralis* (Boisd.) to Gamma Radiation and the Entomopathogenic Fungus *Metarhizium* anisopliae. Egyptian Journal of Biological Pest Control 26 (1) 127-137.

Federici B A. (1981). Mosquito control by the Fungi Culicinomyces, *Coelomomyces* and *Coelomomyces*. In: Burges HD, editor. Microbial Control of Pests and Plant Diseases 1970–1980. 29:555–572.London, Academic Press.

Ferron P. (1978). Biological control of insect pests by entomogenous fungi. Annual review of Entomology. Volume 23, pp 409-442.

Gabarty, S.M.; El-Sonbaty, A.A. and Ibrahim (2013). Synergistic effect of gamma radiation and entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the humoral immune enzyme response in cotton leaf worm *Spodoptera littorals* (Boisd)J. Biol. Sci., Acad. Egypt., 6 (3), pp. 1-10

Georghiou, G. P., and R. Mellon. (1983). Pesticide resistance in time and space, pp. 1-46 In G. P. Georghiou and T. Saito IEds.J. Pest resistance to pesticides. Plenum, New York.

Gerberg, E. (1979). *Manual for mosquito rearing and experimental techniques*. American Mosquito Control Association, Inc.

Goddard, M.R. (2008). Quantifying the complexities of saccharomyces cerevisiae's ecosystem engineering via fermentation.Ecology, 89(8): 2077-2082.

Goddard, M.R. (2008). Quantifying the complexities of saccharomyces cerevisiae's ecosystem engineering via fermentation.Ecology, 89(8): 2077-2082.

Hajek, A. and St. Leger, R. (1994). Interactions between fungal pathogens and insect hosts. Annual review of entomology, 39(1), 293- 322.

Kanzok, S. M., and Jacobs-Lorena, M. (2006). Entomopathogenic fungi as biological insecticides to control malaria. TRENDS in Parasitology, 22(2), 49-51.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685. doi:10.1038/227680a0

Medlock JM; Shuttleworth H; Copley V; Hansford KM and Leach S (2012). Woodland biodiversity management as a tool for reducing human exposure to Ixodes ricinus ticks: A preliminary study in an English woodland. J Vect Ecol. 2012, 37 (2): 307-315. 10.1111/j.1948-7134.2012.00232.x.

Mullen GR and Durden L (2009). Medical and Veterinary Entomology, Mosquitoes (Culicidae) Woodbridge A. Foster and Edward D Walter. Vol. 2. Elsevier, Burlington, pp. 207–260.

Nauen, R. (2007). Insecticide resistance in disease vectors of public health importance. *Pest management science*, 63(7), 628-633.

Rattner, D. (2009). Humanização na atenção a nascimentos e partos: breve referencial teórico. Interface – Comunic., Saude, Educ., v.13, supl.1, p.595-602.

Rawani A, Haldar K M, Ghosh A and Chandra G. (2009). Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research* 105(5): 1411–1417.

Reiter P. (2001). Climate Change and Mosquito-Borne Disease. Environmental Health Perspectives 109 (Suppl. 1): 141—161.

Rivero, A., Vezilier, J., Weill, M., Read, A. F., & Gandon, S. (2010). Insecticide control of vector-borne diseases: when is insecticide resistance a problem?. *PLoS pathogens*, 6(8), e1001000.

Roberts, D.W. and R.A. Humber. (1981). Entomogenous fungi. In Biology of Conidial Fungi (G. T. Cole and W. B. Kendrick, eds.), vol. 2, pp. 201-236. Academic Press, New York.

Sahayaraj, K. and Borgio, J.F. (2010). Virulence of entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin on seven insect pests. Indian Journal of Agricultural Research, 44(3), 195-200.

Scholte, E. J.; Knols, B.; Samson, R. A. and Takken, W. (2004). Entomopathogenic fungi for mosquito control: a review. Journal of Insect Science, 4(1), 19.

Seye, F.; Ndiaye, M.; Faye, O. and Afoutou, J. M. (2013). Evaluation of entomopathogenic fungus *Metarhizium anisopliae* formulated with suneem (neem oil) against *Anopheles gambiae sl* and *Culex quinquefasciatus* adults. Malaria Chemotherapy Cont Elim, 1(6).

Spikes, J. D. and MacKnight, M. L. (1970). Dye-sensitized photooxidation of proteins Ann. N. Y. Acad. Sci. 171,149.

Sree, K. S. and Joshi, H. (2015). Comparative Account of Generalist and Specialist Species of the Entomopathogenic Fungus, *Metarhizium*. In *Biocontrol of Lepidopteran Pests* (pp. 161-167). Springer International Publishing.

ARABIC SUMMARY

التأثيرات البيوكيميائية *لبيوفاريا بسيانا* و *متارزيم انزوبلى* علي الطور اليرقي الثالث لحشرة *كيولكس بيبنز* (ذوات الجناحين: كيوليسيدي)

محمد على محمود عبده* ،داليا عبد البديع سالم، فاطمة ابراهيم عبد الله، نادية لطفي ديوان

قسم علم الحشرات - كلية العلوم – جامعة عين شمس – القاهرة – مصر

تم اختبار التأثيرات البيوكيميائية لفطرين متخصصين في اصابة الحشرات (*بيوفاريا بسيانا* و *متارزيم انزوبلى*) في هذه الدراسة المقدمة في الطور اليرقي الثالث لبعوض *كيولكس بيبنز* فى المختبر.

أظهرت النتائج انخفاض معنوي في المحتوى البروتينى الكلي للطور اليرقي للبعوض المعالج بالفطرين بالمقارنة بالطور اليرقي للعينات المرجعية.

أظهرت الدراسات البيوكيميائية باستخدام التفريد الكهربى للبروتينات (SDS-PAGE) ان هناك انخفاض في عدد الحزم البروتينية لليرقات المعالجة بالفطرين.

واقترحت هذه التفسيرات أن السموم التي يفرزها الجراثيم تسببت في أضرار في بروتينات اليرقات، مما يؤدي إلى موتها.

والاستنتاج أن مسببات الأمراض الفطرية هامة في مجال المكافحة البيولوجية الطبيعية ضد العديد من الحشرات وغيرها والمفصليات وكثيرا ما تسبب الأوبئة الي تقلل عدد كبير من الآفات. وتوصي هذه الدراسة استخدام *بيوفاريا بسيانا* و *متارزيم انزوبلى* كعوامل مكافحة بيولوجية ليرقات البعوض *كيولكس بيبنز* بشكل آمن من أجل قمع هذاه الحشره الطبية ومن اجل الصحة العامة.