Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www. jppp.journals.ekb.eg

Biological Control of *Pectinophora gossypiella* (Saunders) Using Spores and Supernatants of some Entomopathogenic Fungi

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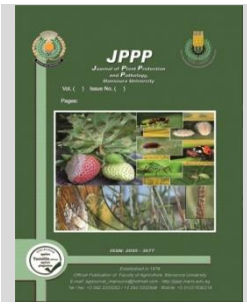

ABSTRACT

The spores and supernatants of some fungal isolates; *Paecilomyces violacea*, *Trichoderma harzianum* and *Beauveria bassiana;* were evaluated to control the pink bollworm, *Pectinophora gossypiella* biologically at Plant Protection Research Institute, Sharkia branch, (ARC). All fungi were isolated from soil and dead pink bollworm larvae. *B. bassiana* was the most active isolate caused the greatest larval and pupal mortality, also the highest deformed adult, also, the lowest numbers of eggs and hatchability percentage. Protease and chitinase produced from *B. bassiana*. strain had high effects on percent of larval mortality, comparing with control. Identification of *Beauvaria* sp. was assured by using 18s rRNA with accession number MT644472.1.

Keywords*: *Paecilomyces violacea*, *Trichoderma harzianum*, *Beuvaria bassiana* and *Pectinophora gossypiella

INTRODUCTION

The most destructive pest of cotton, *Gossypium* spp is the pink bollworm (PBW), *P. gossypiella* (Saunders) which covering the cotton fields all over the world (Henneberry, 2007). Biological control is considering as an alternative method to alleviate the use of harmful chemical pesticides. Entomopathogenic fungi play a vital role in the biopesticides market; *B. bassiana* considering about 50% of registered the most notable microbial biopesticides for its insecticidal activity while it is non-pathogenic to non-target pests. (Dhawan and Joshi, 2017; Mascarin *et al*., 2019 and Mondal *et al*., 2019). *B. bassiana* spores in the hydrophobic cuticle of the host grow into an infectious phase and release solubilizing enzymes causing damage to its cuticle, which contains chitin fibrils imbibed in a matrix of proteins, lipids and N-acylcatecholamines subsequently secretes chitinase, protease and lipase enzymes which accountable for the pathogenesis (Dhawan and Joshi, 2017).

In this point forward, the microbial products; second generation of biopesticides includes naturally occurring compounds by microorganism's excretion or their synthetic analogs. It has become an emerging field of research (Subbanna *et al*., 2018). Enzymes which concerning with cell wall degrading are much likable to be used as biopesticides. They characterized by specificity, stability and extremely fast labor under moderate temperatures and pressures. So, they are considered safe and eco-friendly (Cologna *et al*., 2018).

The present work aim to study the biological activity of some fungal isolates on some biological aspects of *P. gossypiella*. The percent of larval mortality also estimated under the influence of cuticle degrading enzymes.

MATERIALS AND METHODS

I-Rearing technique:

Fourth instar larvae of *P. gossypiella* were collected from infested cotton bolls in Sharkyia province, Egypt and reared at the Bollworms Research Department laboratory, Plant Protection Research Institute, (ARC), Giza, Egypt, for several generations. The neonate larvae were transferred into glass tubes (7 × 2.5 cm) containing about 4g artificial diets incubated at constant temperature of 26 ± 1 °C and 75 ± 5% RH. The diet for maintaining laboratory colony prepared according to (Amer and El-Sayed, 2015).

Moths that emerged were kept under the previously mentioned conditions and supplied with sucrose solution (10%) then mating, females allowed to lay their eggs in glass cage and covered with muslin. The dead larvae were stored in in refrigerator until needed (Mahfouz and Abou El-Ela, 2011).

II-Microbiological analysis

Fungi isolation technique

Isolation of microorganisms associated with the dead PBW larvae, each individual was examined through 24-72 h from the time of storage under aseptic conditions. The larvae were sterilized by emerged in 2% sodium hypochlorite for few min. then washings with sterile distilled water several times (Crecchio and Stotzky, 2001). Sterilized larvae were dried up by sterilized tissues or filter papers, then transferred into a mortar and macerated with a pestle under sterilized conditions then diluted and plated on Czapek-Dox agar medium for growth, incubating at 30°C for 5-7 days. While, isolation from soil was carried out according to the methods described by Johnson *et al.* (1959). Incubated plates were inspected daily to clear the colonies growth then purified and stored on slants of Czapek-Dox agar media in refrigerator.

Screening the mortality effect of fungi on *P. gossypiella*

Spores were obtained by washing the slants (7-days old) of tested isolate (Dulmage *et al*., 1971 and Mohd- Salleh and Lewis, 1983), then inoculated a 100 ml of Czapek-Dox agar medium (Oxoid, 1982) composed of (g/l): 2.0 $NaNO_3$, 1.0 KH_2PO_4, 0.5 $MgSO_4 \cdot 7H_2O$, 0.5 KCl, 20 sucrose and 20.0 agar-agar dissolved in one liter of tap water, pH 5.0 in a flask with each suspension. The inoculated broth medium was incubated at 30°C for 7 days, while metabolites were obtained by filtration. Spore suspension and filtrate of all fungal isolates

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DOI: 10.21608/jppp.2020.108838

were tested for their mortality effect and on biological aspects of *P. gossypiella*.

Bioassay

Two milliliters from spore suspension or supernatant were mixed with the artificial diet in each dish; on the other hand, control diet was mixed with water only. Replicate each treatment 3 times. Batches of twenty newly hatched larvae were transferred to the treated Petri dishes after about 30 min from mixing in the diet. The treated Petri dishes were incubated at the previous conditions. After one day of feeding, dead and alive larvae were counted. The mortality percentages were calculated while corrections of the mortality data were carried out according to Abbott (1925).

The alive larvae of each treatment were singly transferred to glass tubes containing about 4 g of untreated diet then covered with a clean cotton piece and held under the same previous conditions. Larvae were examined every day to record the larval duration and mortality. Pupae were transferred individually to other tubes and incubated until moth emergence then pupal duration, adult emergence percentage; sex ratio (for females) and deformed adults percentage were calculated.

Emerged moths from each treatment were sexed and caged in pairs while eggs deposited on strips of muslin cloth hanged in the Chimney cages. Forty pairs were used from each treatment under the previously mentioned rearing conditions. A piece of cotton soaked in 10% sugar solution was hanged inside the jars near its upper opening for moth feeding and changed by new one daily. The upper openings of cages were covered by muslin cloth followed by a tightly secured paper with rubber bands.

The biological parameters such as preovipositional, ovipositional, postovipositional period, deposited eggs number, males and females longivity were examined daily. To record percent of hatchability, the deposited eggs were collected daily then transferred to a glass jar and incubated at the same conditions.

Characterization of most potent fungal isolate

Identification of isolated fungi by light microscope

The developed fungal colonies were daily examined and the purified fungi were identified to the species level. The identification of fungal genera and species was carried out by the following universally accepted keys for identification of the different isolates. Morphology of colony shape, height and the aerial hyphae color as well as the color of base, growth rate, margin characteristics, surface texture and depth of growth into the medium. Tests were contrasted with an ordered key for the genus *B. bassiana* EA1 (Rifai, 1969).

Molecular characterization (sequence of 18S rRNA gene of DNA)

Sequence of 18S rRNA gene of DNA of fungi was done at the Unit of Molecular Biology at (National Biolab for Trad Dokki Giza Egypt) (Figs. 1 and 2). Molecular characterization involved the following steps according to the protocol adopted by Woese and Fox (1977) and Abdel-Baky and Abdel-Salam (2003).

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Beauveria bassiana strain EA1 small subunit ribosomal RNA gene, partial sequence	2372	2372	100%	0	100.00%	MT644472.1
Beauveria bassiana strain HZBB160701 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S large subunit ribosomal RNA gene, partial sequence	2372	2372	100%	0	100.00%	MH521027.1
Beauveria bassiana isolate 1577 18S ribosomal RNA gene, partial sequence	2372	2372	100%	0	100.00%	JQ861945.1
Beauveria bassiana isolate 1576 18S ribosomal RNA gene, partial sequence	2372	2372	100%	0	100.00%	JQ861944.1
Beauveria bassiana isolate 1573 18S ribosomal RNA gene, partial sequence	2372	2372	100%	0	100.00%	JQ861943.1
Beauveria bassiana isolate ARSEF2991 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	2372	2372	100%	0	100.00%	EU334676.1
Beauveria bassiana strain SZY2 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, 28S ribosomal RNA gene, and 28S-18S ribosomal RNA intergenic spacer, complete sequence	2368	2368	100%	0	99.92%	MN494090.1
Beauveria bassiana isolate 1572 18S ribosomal RNA gene, partial sequence	2368	2368	100%	0	99.92%	JQ861942.1
Beauveria bassiana strain STB 28S-18S rRNA intergenic spacer, partial sequence; and 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 28S ribosomal RNA gene, complete sequence	2368	2368	100%	0	99.92%	JF429894.1
Beauveria bassiana isolate DAOM216540 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	2368	2368	100%	0	99.92%	EU334679.1
Beauveria bassiana isolate DAOM195005 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	2368	2368	100%	0	99.92%	EU334677.1
Beauveria bassiana isolate INRS-CFL 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	2368	2368	100%	0	99.92%	EU334674.1

Figure 1. 18S ribosomal RNA gene of *B. bassiana* MT644472.1

Alignments (Sequence of *Beauveria bassiana JQ977753.1*)

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Query 12   CATGTCT-AGTAT-AGCAATTATACAGCGAAACTGCGAATGGCTCATTATATAAGTTATC 69
           ||||||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 5    CATGTCTAAGTATAAGCAATTATACAGCGAAACTGCGAATGGCTCATTATATAAGTTATC 64
Query70    GTTTATTTGATAGTACCTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGC 129
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct65    GTTTATTTGATAGTACCTTACTACTTGGATAACCGTGGTAATTCTAGAGCTAATACATGC 124
Query130   TGAAAATCCCGACTTCGGAAGGGAGGTATTTATTAGATTAAAAACCAATGCCCTCTGGGC 189
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Query190   TCCTTGGTGATTCATAATAACTTTTCGAATCGCACGGCCTTGCGCCGGCGATGGTTCATT 249
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Sbjct185   TCCTTGGTGATTCATAATAACTTTTCGAATCGCACGGCCTTGCGCCGGCGATGGTTCATT 244
Query250   CAAATTTCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTCGCAACGGG 309
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Sbjct245   CAAATTTCTTCCCTATCAACTTTCGATGTTTGGGTATTGGCCAAACATGGTCGCAACGGG 304
Query310   TAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAA 369
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Sbjct305   TAACGGAGGGTTAGGGCTCGACCCCGGAGAAGGAGCCTGAGAAACGGCTACTACATCCAA 364
Query370   GGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGATTCGGGGAGGTAGTGACAATAAATA 429
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Sbjct365   GGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGATTCGGGGAGGTAGTGACAATAAATA 424
Query430   CTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAATCTCTTAACG 489
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Sbjct425   CTGATACAGGGCTCTTTTGGGTCTTGTAATTGGAATGAGTACAATTTAAATCTCTTAACG 484
Query490   AGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCG 549
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Sbjct485   AGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCG 544
Query550   TATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGT 609
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Sbjct545   TATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGGCTGGCCGGT 604
Query610   CCGCCTCACCGCGTGTACTGGTCCGGCCGGGCCTTTCCCTCTGTGGAACCTCATGCCCTT 669
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct605   CCGCCTCACCGCGTGTACTGGTCCGGCCGGGCCTTTCCCTCTGTGGAACCTCATGCCCTT 664
Query670   CACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGCTCCAGGCAG 729
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Sbjct665   CACTGGGTGTGGCGGGGAAACAGGACTTTTACTTTGAAAAAATTAGAGTGCTCCAGGCAG 724
Query730   GCCTATGCTCGAATACATTAGCATGGAATAATAAAATAGGACGCGTGGTTCTATTTTGTT 789
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Query790   GGTTTCTAGGACCGCCGTAATGATTAATAGGGACAGTCGGGGGCATCAGTATTCAATTGT 849
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Query850   CAGAGGTGAAATTCTTAGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGAT 909
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Sbjct845   CAGAGGTGAAATTCTTAGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGAT 904
Query910   GTTTTCATTAATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTC 969
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Query970   TTAACCATAAACTATGCCGACTAGGGATCGGACGATGTTATTTTTTGACGCGTTCGGCAC 1029
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Sbjct965   TTAACCATAAACTATGCCGACTAGGGATCGGACGATGTTATTTTTTGACGCGTTCGGCAC 1024
Quer1030   CTTACGAGAAATCAAAGTGCTTGGGCTCCAGGGGGAGTATGGTCGCAAGGCTGAAACTTA 1089
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjc1025   CTTACGAGAAATCAAAGTGCTTGGGCTCCAGGGGGAGTATGGTCGCAAGGCTGAAACTTA 1084
Quer1090   AAGAAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACAC 1149
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjc1085   AAGAAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACAC 1144
Quer1150   GGGGAAACTCACCAGGTCCAGACACAATGAGGATTGACAGATTGAGAGCTCTTTCTTGAT 1209
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjc1145   GGGGAAACTCACCAGGTCCAGACACAATGAGGATTGACAGATTGAGAGCTCTTTCTTGAT 1204
Quer1210   TTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGC 1269
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjc1205   TTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGC 1264
Quer1270   GATAACGAACGAGACCTTAACCTGCTAAATAGCCTGTATTGCTTTGGCAGTACACCGGCT 1329
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjc1265   GATAACGAACGAGACCTTAACCTGCTAAATAGCCTGTATTGCTTTGGCAGTACACCGGCT 1324
Quer1330   TCTTAGAGGGACTATCGGCTCAAGCCGATGGAAGTTTGAGGCAATAACAGGTCTGTGATG 1389
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjc1325   TCTTAGAGGGACTATCGGCTCAAGCCGATGGAAGTTTGAGGCAATAACAGGTCTGTGATG 1384
Quer1390   CCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGGAGCCAGCGAGTACTT 1444
           ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjc1385   CCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGGAGCCAGCGAGTACTT 1439
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Fig. 2. 18S ribosomal RNA gene, partial sequence; internal transcribed spacer1 and 1.5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Screening of lipase, protease and chitinase produced by *Beauvaria*

In vitro

Fungal culture (7-days old) used as a standard inoculant. At the end of incubation period for each enzyme, the fungal cultures were filtered and the clear supernatants were considered the source of crude enzymes (lipase, protease and chitinase) (Reda *et al*., 2013).

The most potent isolate of *B. bassiana* was screened for lipase, protease, and chitinase production according to clearing zone technique using Dox-yeast extract-tributyrin agar (Elwan *et al*., 1977); Dox agar with replacing of NaNO3 by 0.2% gelatin (Ammar *et al*., 1991) and chitin media which consists of (g/l): colloidal chitin, 0.5; yeast extract, 0.5; $(NH_4)_2SO_4$, 1.0; $MgSO_4$. $7H_2O$, 0.3; K_2HPO_4, 1.36; agar-agar, 20 (Rajamanickam *et al*., 2012), respectively.

In vivo

The filtrates of *B. bassiana* after 7 days incubation at 30°C for lipase and protease while 14 days at 30°C for chitinase media were obtained and treated with PBW to evaluate the larval mortality.

Statistical analysis

Obtained results were analyzed according to Little and Hills (1975), using CoStat computer program Cohort Software, P. O. Box 1149, Berkeley CA 9471 (CoStat Statistical Software, 2005).

RESULTS AND DISCUSSION

Twelve fungal isolates from naturally dead larvae of the PBW and soil were preliminary bio assayed for their pathogenicity against newly hatched larvae of the *P. gossypiella*. The identification of most effective isolates by morphological and biochemical tests indicated that *P. violacea, T. harzianum* and *Beauvaria bassiana* were the most potent.

Data in Table (1) illustrated the effect of fungal isolates on mortality percentage of larval and pupal and also adult emergence and deformed adults. The analysis variance one way ANOVA test indicated that all fungal isolates showed highly significant difference on larval mortality, pupal mortality, adult emergence and deformed adult percentages. The highest larval mortality was recorded to *B. bassiana* (68.96 and 60.72%) for its spore suspension and metabolites, respectively than the control (4.93%). The pupal mortality showed high significant effects for the three isolates and *B. bassiana* was the highest effective against pupal mortality (16.54 and 12.24%), respectively, for their spores and metabolites compared with control (0%).

Table 1. Effects of some fungi on larval, pupal and adult stages of *P. gossypiella*.

Isolates	larval mortality percentage	pupal mortality percentage	adult emergence percentage	deformed adult percentage
P. violacea s.s.	48.19c	9.22c	83.60d	7.17c
P. violacea f	41.23d	6.53d	87.14b	6.32d
T. harzianum s.s	59.77b	12.08b	80.63e	7.28c
T. harzianum f	48.09c	8.34c	84.97c	6.68d
B. bassiana s.s	68.96a	16.54a	74.70f	8.75a
B. bassiana f	60.72b	12.24b	79.88e	7.87b
Control	4.93e	0e	100a	0e
p	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***
LSD 0.05	1.15	0.98	1.19	0.41

Same letters means non-significant effect while different letters means significant effect
***** means very highly significant effect**
s.s. spore suspension of each isolate, f filtrate of each isolate

Concerning adult emergence percentages in Table (1) the adult emergence percent of larva treated with spore and metabolite of *B. bassiana* was 74.70 and 79.88% whereas the control was 100%. In the same trend, the spores of *B. bassiana* caused deformed adult (8.75%) and its metabolite caused (7.87%) compared with control (0%). finally, the results evident that *B. bassiana* was the most potent isolate than *P. violacea* and *T. harzianum* in controlling the serious cotton pest, the pink bollworm.

Regarding to table (2) there were highly significant of the three isolates even if with spore or filtrate in all the developmental period of survived larvae except in male longevity exhibited moderate significant. The three isolates shortened the larval and the pupal duration. Longevity of emerged females from larvae fed on the isolated fungi demonstrated that the influence on the three reproductive periods (pre-oviposition, oviposition, and post-oviposition). The spore of *B. bassiana* is the most virulence on female longevity it recorded (18.88) days than control (20.11). On the same trend, longevity of males shortened in case of spore suspension of *B. bassiana*.

Table 2. Effect of some fungi on duration of different stages of *P. gossypiella*.

Isolates	Larvae (days)	Pupa (days)	Female longevity (days)				Male longevity (days)
			Preovi position	Ovi position	Post oviposition	Total	
P.violacea s.s.	14.86cd	8.40c	2.33b	11.43d	5.55c	19.31d	18.82bc
P. violacea f	15.15bc	9.21ab	2.43a	11.73b	5.62b	19.79b	19.11bc
*T. harzianum*s.s	14.35e	8.31c	2.23c	11.34e	5.42d	19.00a	18.90bc
T. harzianum f	15.23b	9.14ab	2.44a	11.63c	5.53c	19.51c	19.32ab
B. bassiana s.s.	14.07e	8.18c	2.24c	11.24f	5.40d	18.88e	18.46c
B. bassiana f	14.78d	9.03b	2.33b	11.44d	5.51c	19.28d	19.19b
Control	15.74a	9.72a	2.43a	11.85a	5.83a	20.11a	19.91a
p	<0.0001 ***	0.0003 ***	<0.0001 ***	<0.0001 ***	< 0.0001 ***	<0.0001 ***	0.0079 **
LSD 0.05	0.32	0.56	0.05	0.04	0.05	0.14	0.63

Same letters means non-significant effect while different letters means significant effect
***** means very highly significant effect s.s. spore suspension of each isolate, f filtrate of each isolate**

The tabulated results in Table (3) observed the impact of the spore suspension and filtrate of *P. violacea*, *T. harzianum* and *B. bassiana* on some adult biological aspect; sex ratio, hatchability percentages and number of deposited eggs. The data proved that all fungal isolates demonstrated highly significant action in adult biological aspect mentioned above in comparable to control.

Fortunately, the spore suspension of *B. bassiana* and its filtrate induced a reduction in egg number (214.12 and 233.46) lay by *P. gossypiella* moths, respectively, compared

with control (294.06). Also hatchability percentage decreased by *B. bassiana* spore suspension and filtrate (70.06) and (73.91), respectively, compared with control (93.28). The results are supported by Abd-ElAzeem *et al*., (2019) detected the highly significant effect of *Acremonium* sp. on controlling larvae of *E. insulana* in egg number and hatchability percent.

Table 3. Effect of some fungi on sex ratio, fecundity and hatchability of *P. gossypiella*.

Isolate	♀sex ratio percentage	Eggs no.	Hatchability percentage
P. violacea s.s.	50.48	248.11c	80.33b
P. violacea f	48.37	255.24b	82.97b
T. harzianum s.s	50.64b	242.8d	78.29bc
T. harzianum f	51.45a	252.24bc	80.02b
B. bassiana s.s.	49.50c	214.12f	70.06d
B. bassiana f	50.59b	233.46e	73.91cd
Control	50.38b	294.06a	93.28a
p	<0.0001 ***	<0.0001 ***	<0.0001 ***
LSD 0.05	0.49	4.51	5.02

Same letters means non-significant effect while different letters means significant effect

***** means very highly significant effect**

s.s. spore suspension of each isolate, f filtrate of each isolate

The most active isolate fungus which induced the highest larval mortality of *P. gossypiella* was *B. bassiana* EA1 so that, it selected for further study. Molecular characterization confirmed by 18S rRNA sequencing. Figure (1) showed there were homologs 100% of the selected eluted PCR products sequencing with the sequence of *B. bassiana* MT644472.1. Regarding to the phylogenetic tree the position of *B. bassiana* MT644472.1 was observed which erect upon the partial 18S rRNA gene sequences from the evolutionary distance matrix.

Screening of the *B. bassiana* MT644472.1 for production of lipase, protease and chitinase enzymes

B. bassiana MT644472.1 produced high activities of protease and chitinase enzymes but it exhibited nil activity of lipase by using clearing zone technique *in vivo*.

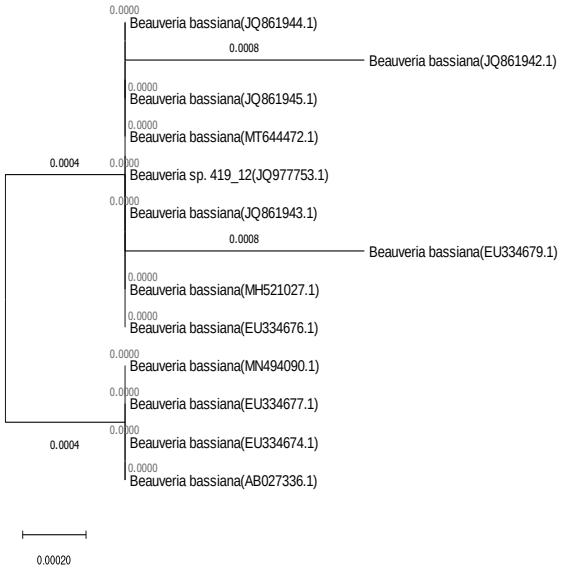

Fig. 3. Phylogenetic dendogram of different fungal isolates accessions revealed by average linkage cluster analysis based on 18S rRNA partial sequence

The data in Figure (4) represented the screening of *B. bassiana* MT644472.1 filtrates separated or mixed (1:1 v/v) against the pink bollworm larvae. The data revealed that the highest mortality percentage in *P. gossypiella* was achieved when mixed *B. bassiana* MT644472.1 protease and chitinase filtrates was (78.37%) while the mortality percentages for protease and chitinase each alone were (65.43%) and (63.46%), respectively, compared with control (5.56).

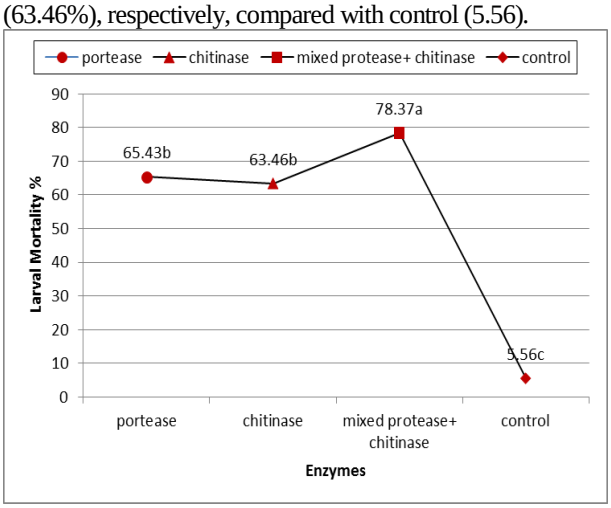

Fig. 4. Screening of *B. bassiana* filtrates against P. gossypiella (*In vivo*)

These results are in harmony with Cristina and Gheorghe, (2017) exacted the efficacy of cuticle-degrading enzymes produced by *B. bassiana* and *Paecilomyces* sp. hydrolyzed (protein, chitin and lipid) through the infection process causing a high larval mortality percentages against *E. insulana*. On the other hand, Abd-ElAzeem *et al*. (2019) demonstrated that *Acremonium* sp and *Paecilomyces variotii* have the capability to produce protease enzymes which act as cuticle degrading enzyme, so it can play an important role in the control of *E. insulana*. Finally, Alvesa *et al*. (2020) found that cocktail of cuticle degrading enzymes caused higher larval mortality than each enzyme alone against *Phereoeca uterella*

CONCLUSION

Beauvaria bassiana proved that it can be tolerant to control the pink bollworm *P. gossypiella* due to its ablity to secrete protease and chitinase (cuticle degrading enzymes), so it can insert at IPM programs.

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المكافحة الحيوية لدودة اللوز القرنفلية باستخدام جراثيم ورشيح بعض الفطريات اللمرضة للحشرات

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تم دراسة النشاط البيولوجي لجراثيم ورشيح بعض العزلات الفطرية (البيسلوميسس فيوليسي و التريكودرما هارزيانم و البيوفاريا باسيانا) ضد ديدان اللوز القرنفلية في معهد بحوث وقاية النباتات - فرع الشرقية- مركز البحوث الزراعية. كل الفطريات تم عزلها من التربة ويرقات ديدان اللوز الميتة. البيوفاريا باسيانا اعطى أعلى نسب موت لليرقة والعذارى واعلى نسب تشوه للفراشات المشوهة وقلل عدد البيض وايضا نسبة الفقس. انزيمي البروتيز والكيتنيز المنتجين من البيوفاريا باسيانا لهما تأثيرا كبيرا على نسبة موت اليرقة مقارنة بالكنترول. تعريف البيوفاريا تم تأكيدة بتحليل الحمض النووي وتم تسجيله في بنك الجينات برقم MT644472.1