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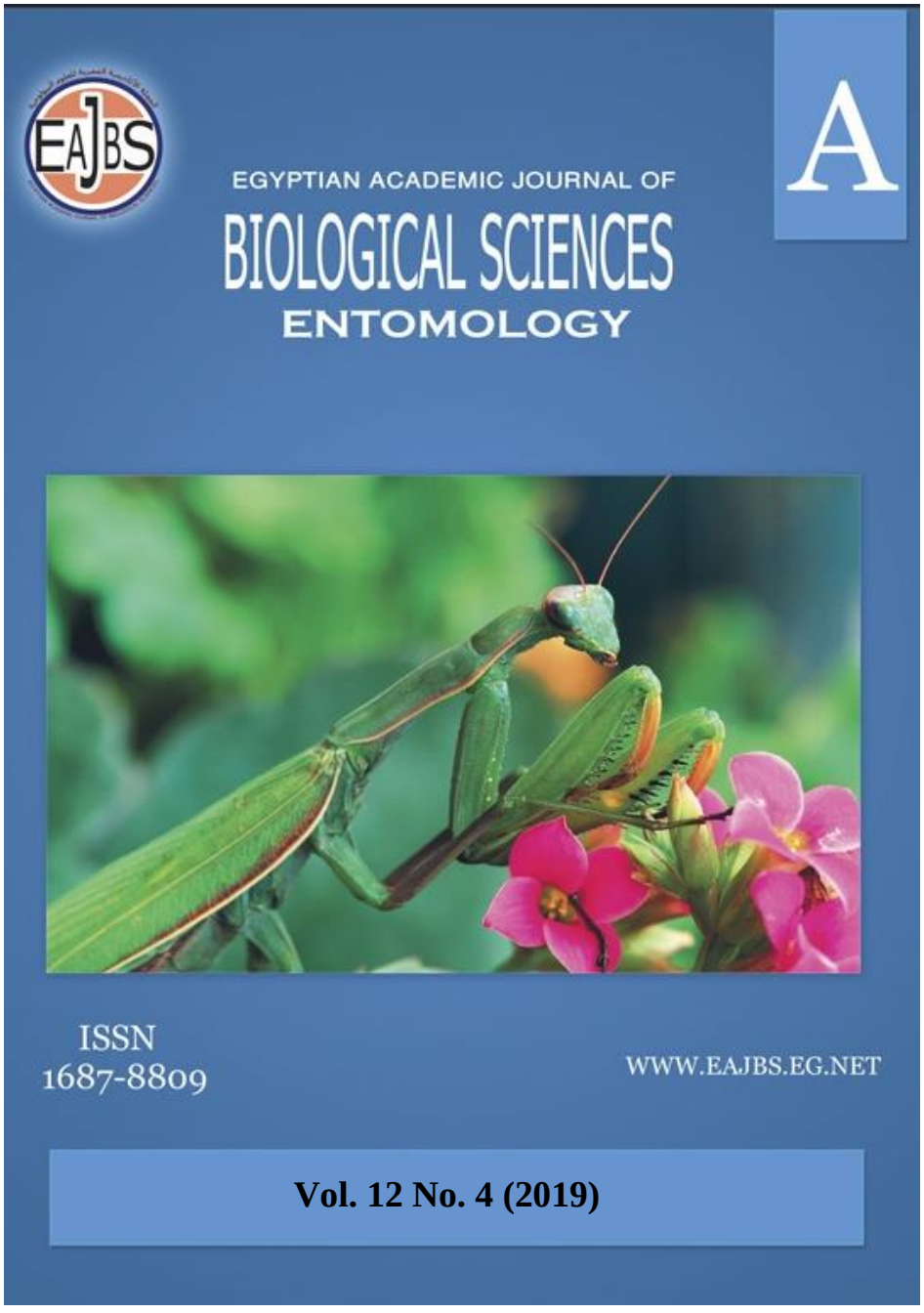

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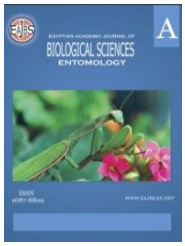

Evaluation of the Effectiveness of Some Entomopathogenic Fungi on the Greater Wax Moth Larvae, *Galleria mellonella* (L.) (Lepidoptera: galleriidae)

Shoukry, I. F[1], Ahmed,F.A[1]Khater, K. S. [1] El-lakwah, S .F.[2] and Abd-Elmonem, H. M.[2]

1-Faculty of Science - Zagazig University
2-Plant Protection Research Institute, Dokki, Giza, Egypt

Email: hendabdelmonem1111@gmail.com

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ABSTRACT

The present study designed to investigate the effect of three entomopathogenic fungi (EPF), *Beauveria bassiana* (Biovar), *Trichoderma album* (Biozed) and *Metarhizium anisopliae* (Bioranza) on greater wax moth *Galleria mellonella* larvae. The results revealed that the LC_{25} values of *T. album*, *M. anisopliae* and *B. bassiana* against *G. mellonella* larvae were 14.08, 44.20 and 57.82 ppm, respectively. The LC_{50} were 104.03, 252.55 and 389.05 ppm, respectively for the same biocides. *T. album* was the most toxic biocide for controlling *G. mellonella* larvae, While *B. bassiana* was the least toxic one. The slope of the tested compounds was 0.78, 0.89 and 0.81 for *T. album*, *M. anisopliae* and *B. bassiana*, respectively. The toxicity index of the tested compounds was 24.35 & 26.74 & 31.85 and 41.18, respectively. On the other hand, the relative potency of the three tested compounds at LC_{25} and LC_{50} were 1.31 & 1.54 and 4.11 & 3.74 fold for both *M. anisopliae* and *T. album* compared to 1.00 fold in case of *B. bassiana*. The results showed that the cumulative mortality percentages of *G. mellonella* larvae treated with the three products can be arranged according to the most efficient compounds 47.61, 41.52 and 39.67%, respectively for *T. album*, *M. anisopliae* and *B. bassiana* compared to control. Also, the results indicated that three tested biocides have a significant effect on some biological aspects of *G. mellonella* larvae. *B. bassiana* was the most effective biocide on larval and pupal duration, pupal mortality, pupal weight and sex ratio percentages. While, treatment with *M. anisopliae* reduced male longevity, and the malformation percentages recorded as 27.35% compared to control. But, *T. album* had the most toxic effect on accumulative larval mortality, pupation percentages, pupal duration and significantly decreased the female sex ratio than control. The three tested biocides had an effect on some biochemical parameters of *G. mellonella* larvae. The activity of amylase enzyme had a significantly decreased effect after five periods of treatment compared to control. A significant increase in relative activity in protease enzyme was recorded after treatment with the three EPF. A fluctuation in the enzyme activity of transaminase enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was estimated. There was also a significant reduction in total protein content. Results cleared that, significantly increased gradually in total lipid up to ten days after treatments. The results indicated that the tested bio-products can be used in honey bee colonies as a strategy to control *G. mellonella* larvae as one of the components of biological control programs.

INTRODUCTION

Galleria mellonella, the greater wax moth or honeycomb moth is well known for its parasitization of honeybees, *Apis mellifera* and their hives through feeding upon pollen and destroying the combs and honey by tunneling into the wax and leaving behind a mass of webs and debris. This species is one of the most devastating and economically important pests of wax in the world (Chang and Hsieh, 1992) and Haewoon *et al*., (1995). Also, it stated that wax is one of the most useful products of honey bees and is used in the pharmaceuticals industry, dentistry and cosmetics. In addition, *G. mellonella* larvae caused the economic loss in honey colonies. (Kwadha *et al.*, 2017) reported that, the larvae caused severe damage in tropical and sub-tropical regions, and are believed to be one of the contributing factors to the decline in both feral and wild honeybee populations. Therefore, most studies have focused on this pest as a model for in vivo studies of toxicology and pathogenicity. Also, other researchers stated that, as an alternative to chemical control, the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* contribute to the natural control of a wide range of insects (Butt *et al*., 2001) also, Steenberg and Kilpinen (2003). The type of toxins produced may also be helpful in defining the mode of action of the EPF (Vey *et al*., 1993).

(Lacey *et al*., 2008) who tested the EPF to control *Bemisia tabaci*. (Barbarin *et al*., 2012) reported that the EPF infect insects have received considerable attention by scientists of their potential for biological control pests. Some pathogenic fungi have restricted host ranges while other fungi species have a wide host range, for example, *B. bassiana* and *M. anisopliae*. Many researchers have focused on the selection of virulent strains for target pests and their development as biological control agents (Amer, El-sayed, Bakheit, Mostafa, & El-sayed, 2008; Amora *et al*., 2010). Other researcher reported that the EPF is potential and environmentally safe method has no effect on non-target invertebrates and plant species in addition to their wide use in the control of many insect pests (Zimmermann, 1993). In Egypt, some researchers found *Beauveria* spp. is considered one of the most common species used in biological control against insect pests (El-Sinary and Rizk, 2007). *B. brongniartii* has a high pathogenic effect against the larvae of *Dendrolimus tabulaeformis* (Jinhua *et al*., 2013). Other researcher tested *Metarhizium* and *Beauveria*, isolates against 3rd instar larvae of *Hoplia philanthus*. And found two isolates of *M. anisopliae* caused 90% mortality post-inoculation (Ansari *et al*., 2004). Four concentrations of the EPF isolates, *B. bassiana* and *T. harzianum* were tested against both larval and pupal stages of *Spodoptera littoralis* within five days post-treatment. *T. harzianum* showed highly larval mortality. However, *B. bassiana* showed moderately mortality for larvae and pupae (Ashraf and El-Katatny, 2007). The genus *Metarhizium* spp. can be used in biological control *Dermanyssus gallinae* and infect their hosts through the cuticle, penetrate them and spread through the body and after the fungus has killed the host, it can grow out of the host cadaver and produce more spores, increasing the chance for others to be infected (Mul *et al.*, 2009). Also, other researchers reported than, several entomopathogenic fungi species of *M. anisopliae*, which is widely used as, *M. anisopliae* and *Beauveria* spp. and reported, *M. anisopliae* are considered the most common EPF species used as biological control agents against *Agriotes* spp. and other insect pests (Ladurner *et al*., 2009). EPF were the most effect on *Ceratitis capitata* larvae by Imoulan and Elmeziane. (2014). In Egypt, it was found that *T. harzianum* liquid filtrate and spores had to produce a large mortality rate and caused significant reduction in adult emergence significant effect on the larvae of *Earias insulana* and *Pectinophora gossypiella* caused more mortality rate for the tested insects (El-Massry *et al*., 2016).The treated *G. mellonella* with *B. caledonica* and *M. anisopliae* caused a significantly increased yeast cell density and increased larval mortality (Namara *et al*., 2017). *Trichoderma* spp. has been widely used as

antagonistic fungal agents against several pests (Verma *et al*., 2007). In Egypt, the treatment of the desert locust, *Schistcereca gregaria* with *M. anisopliae* caused highly specific biopesticides effect. Also, the results showed that the insect enzymes Phenol oxidase activities were fluctuated between increasing and decreasing by fungi infection. Total insect proteins and lipids contents were dramatically declined in all treatments (Elbanna *et al*., 2012). Our experiments have shown that the three tested biocides can be used in bee colonies for control *G. mellonella* larvae as biological control.

The aim of the present work was conducted to throw a spotlight on the toxicology, biological effects and some biochemical aspects of the three Entomopathogenic fungi, *B. bassiana, M. anisopliae* and *T. album* on the greater wax moth *G. mellonella* larvae.

MATERIALS AND METHODS

1. Rearing Technique of *G. mellonella* Larvae:

The greater wax moths were obtained from Plant Protection Research Institute, and reared on a semi-synthetic artificial diet as described by Ibrahim *et al*., (1984). After that kept in laboratory conditions at 28±2^{o}C and 60-70% R.H. for several generations far from any insecticidal contamination. The larvae were reared in semi-synthetic diet layer of 5-7 cm thick is placed in a glass jar (9.40 cm diameters x 1.50 cm high) capacity and covered with plain paper fitted in place with two rubber bands. The alive larvae were supplied with an artificial diet to the developing larvae till pupation. The accumulated faces and debris were cleaned out daily. After pupation, pupae were collected and placed in a wide glass jar until adult emergence. The emerged adults were collected for males and females per each glass jars as well as untreated adults. The deposited eggs were collected daily and transferred to clean the glass jars then incubated at the previous condition to carry out the different experiments.

2. Toxicological Studies:

The efficiency of the three tested biocides Table(1); Biovar, (*B. bassiana*), Bioranza, (*M. anisopliae*) and Biozed, (*T. album*) were estimated against last instar larvae of *G. Mellonella*.The LC_{25}, LC_{50}, slope values, Toxicity Index and Relative Potency were determined. Serial successive concentrations of aqueous solutions for each product were prepared. Each tested of biocide started with double of the recommended, recommended, half recommended and quarter recommended as follows; 400, 200, 100 and 50 ppm concentrations for *M. anisopliae* and *B. bassiana* fungi. Meanwhile, the tested concentrations for *T. album* were as follows; 125, 62.5, 31.24, 15.625 ppm using distilled water. The tested concentrations were prepared using the dipping technique of *G. mellonella* larvae in each concentration for about 30 seconds. Five replicates were prepared for each concentration as well as control, (10 larvae/replicate). Larval mortality percentages were recorded after 72hrs from treatment as well as untreated once. The mean of accumulative mortality percentages of larvae was determined after seven days post-treatment.

Table (1): Tested Biocides (Entomopathogenic fungi)

Biocides	Trade name	Product formulation	Rate of application/100L water	Product source
Beauveria bassiana	Biovar, 10%	Wettable, powder, (WP):	200g	Plant Protection Research Institute Dokki, Giza, Egypt
Metarhizium anisopliae	Bioranza, 10 %		200g	
Trichoderma album	Biozed, 2.5%		250g	

3. Biological Studies:

For studying the biological effects of *B. bassiana*, *M. anisopliae* and *T. album* against *G. mellonella* larvae, the compounds were used at LC_{25}. After larval mortality, the alive larvae were transferred individually to clean tubes (2.5cm x 7.5cm) and kept of 28-30 ºC and 60-70% R.H. The survived larvae were inspected and recorded daily. Also, different biological aspects were recorded as follows; larval mortality, larval duration, pupation percentage, pupal mortality, pupal duration, pupal weight, adult emergence, adult longevity (male and female), sex ratio male/female and adults deformation).

4. Biochemical Studies:

Larval samples used for biochemical assays were collected at one, three, five, seven and ten days post-treatment of *G. mellonella* larvae with LC_{50s} of the tested biocide compounds. Untreated larvae were used as control. The treated samples and control of the last instar larvae were homogenized in distilled water, using a Teflon homogenizer surrounded with a jacket of crushed ice for three minutes. The homogenates were centrifuged at 5000 r.p.m for 30 minutes at 5ºC. The supernatants were immediately assayed for determination of total soluble protein according to Gornall *et al*.(1949), total lipids content (Schmit, 1964), The activities of, transaminases aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity (Reitman and Frankle, 1957), amylase enzyme (Ishaaya and Swiriski, 1976) and protease activity (Tachell *et al.*, 1972).

5. Statistical Analysis:

The mortality percentages *G. mellonella* were corrected according to Abbott's (1925) formula. The LC_{25s} and LC_{50s} and the slope values were determined according to Finney (1971). Toxicity index (T.I) at LC_{25}, LC_{50} levels were determined using (Sun, 1950) equation. Relative potency levels of the tested compounds are expressed as the number of folds was measured according to the method described by Zidan and Abdel- Maged (1988). The proper "p" and $LSD_{0.05}$ value of various treatments was evaluated by the range test ($P \leq 0.05$) was calculated as described by Fisher (1944) and Snedecor (1970).

RESULTS AND DISCUSSION

Toxicological Studies:

The results in Table (2) revealed that the LC_{25} of Biovar, Bioranza and Biozed against *G. mellonella* larvae were 57.82, 44.20 and 14.08 ppm, respectively. The LC_{50} values were 389.05, 252.55 and 104.03 ppm, respectively. Based on LC_{50} values it cleared that, *T. album* was the most toxic biocide for controlling *G. mellonella* larvae. While *B. bassiana* has the least toxic effect. The slope function was recorded as 0.81± 0.19, 0.89± 0.19 and 0.78±0.19, respectively. At LC_{25} the toxicity index for *B. bassiana* and *M. anisopliae* against *G. mellonella* larvae were 24.35 and 31.85 units, respectively but at LC_{50} were 26.74 and 41.18 units, respectively. Meanwhile, toxicity index for *T. album* was 100 units as a standard biocide.

The potency levels of the three tested biocides are expressed as the number of folds at the required toxicity level, compared with the least effective compound against *G. mellonella* larvae. Hence the number of folds representing the relative potency level in potency levels at LC_{25} were 4.11 and 1.31 fold for both of *T. album* and *M. anisopliae*, respectively but at LC_{50} it was 3.74 and 1.54 times for *T. album* and *M. anisopliae*, respectively as toxic as *B. bassiana* fungi, respectively.

These results were supported by Ashraf and El-Katatny (2007) who tested different concentrations of *B. bassiana* and *T. harzianum* against both larval and pupal stages of *Spodoptera littoralis* within five days post-treatment. They reported that *T. harzianum*

showed 80% larval mortality. However, *B. bassiana* showed relatively mortalities in some biological aspects.

Table (2): Toxicity of the three biocide products *B. bassiana, M. anisopliae* and *T. album* against the last instars larvae of *Galleria melonella*.

Tested biocides	Concentrations ppm		Confidence limits of				Slope ±SE	Toxicity Index % at		Relative Potency	
			LC_{25}		LC_{50}						
	LC_{25}	LC_{50}	Lower	Upper	Lower	Upper		LC_{25}	LC_{50}	LC_{25}	LC_{50}
Beauveria bassiana	57.82	389.05	251.737	1032.89	21.91	89.22	0.81 ±0.19	24.35	26.74	1.00	1.00
Metarhizium anisopliae	44.20	252.55	180.30	449.18	16.54	69.62	0.89 ± 0.19	31.85	41.18	1.31	1.54
Tricoderma album	14.08	104.03	68.31	263.07	68.31	263.07	0.78 ± 0.19	100	100	4.11	3.74

S.E. = Standard error

Also, Reda and El-Nemaky (2008) in Egypt studied the biological parameters of the pink bollworm, *Pectinophora. gossypiella* larvae when treated with the biocides; Protecto (*Bacillus thuringiensis*), Biover (*B. bassiana*) and Protecto + Biover. (Ladurner *et al*., 2009) who recorded more than 700 species of EPF *M. anisopliae*, which is widely known and used as *M. anisopliae* and *Beauveria* spp. and considered the most common EPF species used as biological control against *Agriotes* spp. wireworm in potatoes insect pests. The same results were conducted by Vijaykumar *et al*., (2009) who tested the effect of the culture filtrate of *T. harzianum,* against the larvae of all the bollworms.

The efficacy of the EPF *B. bassiana* and *T. album* against the red mite, *D. gallinae* evaluated by Kaoud, (2010). Also, other researcher (Cipriano *et al*., 2011) stated that the effect of EPF against *S. frugiperda* reported the highest mortality rate at LC_{50} after treatment. The treatment of *Myzus persicae* with the LD_{50} of *B. bassiana* at laboratory conditions caused decreased some biological aspects by Zhu *et al.,* (2011). The effect of *B. bassiana* against *Earias insulan* was evaluated. The accumulated mortalities of *Earias insulana* larvae, after treatment, were represented as the acute toxicity of *B. bassiana* and gave lower larval mortality against *E. insulana* (Hegab and Zaki, 2012). The treated *Agriotes* wireworms, larvae with EPF, *B. bassiana* and *M. anisopliae* caused severe damage to economic important crops (Kleespies *et al.,* 2013). Other researchers stated that *M. anisopliae* caused adhesion, germination, appressorium formation, penetration, colonization of haemolymph, and extrusion and sporulation, *Metarhizium* spp. such as other researcher reported *M. anisopliae* caused more effect against *Diatraea flavipennella* larval period (Jennifer *et al.,* 2014) also Mora *et al*.,(2017). The tested conidial suspension of the fungus *B. brongniartii* has a high pathogenic effect against the puparia of *D. tabulaeformis* and caused a significant reduction in adult emergence.

The slopes of regression lines for *B. bassiana* were also estimated (Jinhua *et al*., 2013). The larvae of *Pectinophora gossypiella* were susceptible to fungi, (*B. bassiana* and *M. anisoplae*) when treating the newly hatched larvae of *P. gossypiella* with different concentrations of both fungi (El-Akad *et al.,* 2016). Treated the larvae of *E. insulana* and *P. gossypiella* using *T. harzianum* spores caused moderately high mortality for insect treatment, respectively (El- Massry *et al*., 2016). Others reported that *B. bassiana* can be used as bio-control agents, the treated of *G. Mellonella* with culture filtrate of *B. caledonica* and *M. anisopliae* showed a significant increased larval mortality (Namara *et al*., 2017).

Biological Studies:

Data presented in Table (3) indicated that, there is no larval mortality recorded in control experiments while, all biocides tested caused significant increase effect between the

tested biocides on *G. mellonella* larvae as compared with control, the highest average percentage of larval mortality (47.61%) was obtained with *T. album* followed by 41.52 % mortality for *M. anisopliae* and 39.67 % for *B. bassiana*, respectively compared with control. The tested biocides caused a significant decrease in larval duration of *G. mellonella* compared with control. The mean larval durations were 5.00, 5.06 and 5.68 days, for *B. bassiana*, *T. album* and *M. anisopliae* fungi, respectively compared with 7.70 days for control larvae. The effect of the three tested products on the pupation percentage of *G. mellonella* was shown in Table (3) statistical analysis indicated that there was a significant decrease between the tested biocides and control. The lowest average of pupation percentage was 83.00 % for *T. album*. While the high one was 95.00 % recorded for *B. bassiana* meanwhile *M. anisopliae* recorded 88.00% as compared with 90.00 % for control. All the tested products caused prolongation and had a significant increase effect on pupal duration in *G.mellonella* larvae compared with control. The lowest pupal duration was 11.95 days for *T. album*. While the highest was 12.56 days in case of *B. bassiana* compared with 9.04 days in control. Also, the tested compounds caused a significant increase in pupal mortality percentage of *G. mellonella* larvae. The highest average pupal mortality percentage 34.44 % was obtained with *B. bassiana* while the lowest average was 23.79 % for *T. album* followed by 29.72 % which recorded for *M. anisopliae* as compared with 7.75 % for control. A significant decrease effect of the tested biocides recorded on the pupal weight of *G. mellonella* larvae. The mean values were as follows; 0.089, 0.093 and 0.91 g for *B. bassiana*, *M. anisopliae* and *T. album* fungi, respectively compared with 0.15 g in control (Table 3). The statistical analysis elucidates a significant reduction effect on adult emergence after treatment with the three tested biocides. The average of adult's emergence was ranged between 65.56, 65.27 and 75.24 % for *B. bassiana*, *M. anisopliae* and *T. album*, respectively compared with 92.25 % in control.

Generally, it was noticed that the three tested fungi caused lower adult emergence than control. There was insignificantly effect in the sex ratio by the three tested biocides compared with control. The results tabulated in the same Table indicated, that the three tested biocides had a significant decrease effect on the *G. mellonella* adult longevity. The lowest mean periods were 8.76 and 4.86 days, respectively for both male and female longevity treated with *M. anisopliae* and *T. album* as compared with 10.73 and 6.04 days, respectively in control.

The present results in Table (3) cleared that, tested biocides had a highly increased significant effect on adult malformation of *G. mellonella* larvae compared with control. The mean of malformation ascending as follows; 27.35 % for *M. anisopliae* followed by 24.86 % for *B. bassiana* and 15.77% for *T. album* compared with 0.00 % in control.

These results were similar to results reported by many authors, (Fuguet and Vey, 2004) tested eleven strains of *B. bassiana*, and a further five species of *Beauveria* sp. of the larvae of *G. Mellonella*. The virulent strains killed 100% of the insects at slightly different rates. Other researchers indicated that the virulence of EPF isolates from *Metarhizium* and *Beauveria*, on 3rd instar larvae of *H. philanthus* beetle was tested. Two isolates of *M. anisopliae* caused 90% mortality post-inoculation. The LT_{50} values for this isolate were estimated by Ansari *et al*., (2004). In Egypt the, biological parameters of P. *gossypiella* newly hatched larvae were affected on some biological aspects when used biocides, there was prolongation in both pupal duration and female adult longevity especially in oviposition period except for with Protecto + Biover treatment (Reda and El-Nemaky, 2008). The tested *B. bassiana* caused different influences on all biological aspects of *E. insulana* which decreasing larval duration, pupal weight, pupation percentage, adult emergence, adult longevity, female fecundity decreased strongly and hatchability percentages comparing with control (Hegab and Zaki, 2012). The effect of *B. bassiana* against the *Ceratitis*

capitata larvae was evaluated. The tested isolates caused a high mortality rate in puparia and a significant reduction in adult emergence (Imoulan and Elmeziane, 2014). *T. harzianum* has been used for bio-control of the different pests of crop plants (Shah and Pell, 2003). The fungus *B. brongniartii* caused a high pathogenic effect against the larvae of *D. tabulaeformis* (Jinhua *et al*., 2013). The same results reported by Berini *et al*., (2015) that, *T. viride* caused mortality of the pupa and larvae of *Bombyx mori*. The results were identical with El-Akad *et al.*, (2016) who, revealed that, changes in the different biological aspects (larval duration, pupation percentage, pupal duration and the percentage of adult emergence) of *P. gossypiella* affected when treating the newly hatched larvae with both of *B. bassiana* and *M. anisoplae.* Also, results agreed with Kimberly *et al.*, (2017) and Mora *et al*.,(2017) they found that the effect of *M. anisopliae* fungi against larvae of *D. flavipennella* caused differences relative the larval period after treatment.

Table (3): Biological effects of three biocide products from entomopathogenic fungi on certain biological aspects of *G. mellonella* larvae.

Treatments	Accumulative larval mortality %	Larval duration (days)	Pupation %	pupal duration (days)	Pupal mortality %	Pupal weight (g)	Adult emergence %	Sex ratio % /female	Adult longevity (day)		Malformation %
									Male	Female	
Control	0.00c	7.70[a]	90.00ab	9.04b	7.75b	0.15a	92.25a	10.00	10.73ab	6.04b	0.00b
Beauvearia Bassiana	39.67b	5.00b	95.00a	12.56a	34.44a	0.089b	65.56b	9.99	11.95a	5.73b	24.86a
Metarehzium Anisopliae	41.52ab	5.68ab	88.00ab	12.53a	29.72a	0.093b	65.27b	9.99	8.76b	5.73b	27.35a
Tricoderma Album	47.61a	5.06b	83.00b	11.95a	23.79ab	0.091b	75.24ab	10.00	11.08ab	4.86c	15.77[a]
P.	0.00	0.006	0.16	0.01	0.00	0.00	0.017	1.00	0.60	0.00	0.001
L.S.D$_{0.05}$	7.89	2.46	10.49	1.18	16.78	0.025	18.49	4.11	2.42	1.25	13.78

P= Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately

Biochemical Studies:

1. Amylase:

Data represented in Table (4) refer to the activity of amylase enzyme in *G. mellonella* larvae at different intervals after one, three, five, seven and ten days recorded significantly decreased, after treatments with the three tested fungi. Data showed that *B. bassiana* treatment caused significant decreased and recorded (949.22, 914.42, 481.24, 226.23 and 310.49 mg glucose/g.b.wt. /min.) after five periods, respectively. In the case of *M. anisopliae* the activity ranged between (508.52, 334.27, 394.01, 177.12 and 165.96 mg glucose /g. b.wt. /min.), respectively. Also, *T .album* were recorded decreased in the activity of amylase enzyme mounted (825.02, 668.48, 595.63, 380.63 and 665.87 mg glucose /g.b.wt./min.) at five periods post-treatment, respectively. Also noticed that *M. anisopliae* caused highly reduction in the activity of amylase enzyme after seven and ten days post-treatment comparing with (1035.27, 1403.84, 1624.11, 792.85 and 866.24 mg glucose /g.b.wt. /min.) in control, respectively after five intervals from treatment.

2. Protease:

The results cleared a general increase in the activity of protease enzyme in *G. mellonella* larvae after treatment with the three EPF. Significant increases in the activity noticed was (77.14, 32.65, 64.38, 52.75 and 75.41 mg tyrosine/min/g.b.wt.) after five periods from treatments, respectively for *B. bassiana*. Meanwhile the activity in enzyme reached to (46.74, 26.45, 32.83, 27.69 and 58.13 mg tyrosine/min/g.b.wt.), respectively after five periods using *M. anisopliae* followed by (74.29, 42.37, 22.75 and 89.76 mg tyrosine/min/g.b.wt.) for *T. album* after one, three, seven and ten days from treatments,

except at five days *T. album* caused decreased reached to (16.37 mg tyrosine/min/g.b.wt.) as compared to (26.54, 10.42, 18.83, 9.76 and 20.31 mg tyrosine/min/g.b.wt.), respectively in control treatment Table(4).

Table (4): Changes in the activities of Amylase and Protease enzymes in *G. mellonella* larvae after treatment with the three biocide products *B. bassiana , M. anisopliae* and *T. album* entomopathogenic fungi after five periods.

Treatments		Amylase (mg glucose /g. b. wt./min./days)					Protease (mg tyrosine/min/g.b.wt.)				
		1day	3 day	5 day	7 day	10 day	1 day	3 day	5 day	7 day	10 day
B. Bassian	SA	949.22b	914.42b	481.24c	226.23c	310.49c	77.14a	32.65b	64.38a	52.75a	75.41b
	RA %	-8.31	-34.86	-70.37	-71.47	-64.16	190.66	213.34	241.90	440.47	271.29
M. Anisopliae	SA	508.522d	334.72d	394.01d	177.12d	156.96d	46.74b	26.45b	32.83b	27.69b	58.13b
	RA %	-50.88	-76.16	-75.74	-77.66	-81.88	76.11	153.84	74.35	183.71	186.21
T. Album	SA	825.02c	668.48c	595.63b	380.63b	665.87b	74.29a	42.37a	16.37c	22.75b	89.76a
	RA %	-21.03	-52.38	-63.33	-51.99	-23.13	179.92	306.62	-10.09	133.09	341.95
Control		1035.27	1403.84a	1624.11a	792.85a	866.24a	26.54c	10.42c	18.83c	9.76c	20.31c
p		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$LSD_{0.05}$		46.88	22.36	21.51	12.47	60.52	7.13	11.73	9.93	9.22	15.36

P= Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately

RA%= Relative activity Increase or decrease than control = (treated – control) ÷ control ×100.

SA= Specific activity g. b. w.t = gram body weight.

3. Transaminase Enzymes (ALT and AST):

Data in Table (5) showed that the activity of alanine aminotransferase (ALT) activity in *G. mellonella* larvae using *B. bassiana, M. anisopliae* and *T. album* fungi caused a highly significant increase in enzyme activity, was ranged from (289.21, 116.56, 207.50, 152.90 and 56.23 µg pyruvate /g.b.wt.) for *B. bassiana* as compared with control. Meanwhile, in case of *M. anisopliae* treatment, the activity was gradually increased after five periods of post-treatment and recorded (91.19, 73.64, 214.90, 273.50 and 228.34 µg pyruvate /g.b.wt.). Also, data cleared that, the activity of *T. album* treatment was (80.74, 101.77, 192.50, 130.19 and 180.90 µg pyruvate /g. b.wt.) as compared with (50.18, 61.53, 139.86, 72.03 and 25.45µg pyruvate /g. b.wt.). On the other hand the aspartate aminotransferase enzyme (AST). The present results in the same table showed that the activity of enzyme was fluctuated between increasing and decreasing by fungi infection. Data revealed that *B. bassiana,* caused significant increase effect in *G. mellonella* larvae as follow; (45.82, 48.28, 89.73 and 153.90 µg pyruvate /g.b.wt.) after all days of treatment, but the activity of enzyme decreased and recorded (10.04 µg pyruvate /g.b.wt.) at ten days post-treatment. Also, *M. anisopliae* showed an increase in the activity ranged between (42.79, 27.35, 21.10, 25.01 and 65.15 µg pyruvate /g.b.wt.) after five periods of post-treatment. Meanwhile, *T. album* showed that the activity of enzyme was gradually significant decreased effect and recorded (12.89, 7.62, 10.57 and 5.69 µg pyruvate /g.b.wt.) at one up to seven days, while, after ten days the activity increased and reached to (52.55 µg pyruvate /g.b.wt.) as compared with control.

Generally, the previously results indicated that, the activity of both ALT and AST in the last instar larvae of *G. mellonella* had fluctuated between increasing and decreasing by fungi infection after treatment with the median lethal concentration after five periods from treatments. The result of this study was accordance with Dadd, (1985) and Genc, (2002) who found that in the laboratory studies were conducted on the greater wax moth larvae using

some fungal strains showed that the treatments greatly affected some of the physiological aspects of insects according to certain researchers from different countries. Carbohydrates are an important source of energy for insects and it caused converted to lipids, and the production of amino acids. Many carbohydrates such as sugars are powerful feeding stimulants. Other researchers (Remia *et al.,* 2008) noticed the reduction of carbohydrates may due to the effect of anti-feedent and increased metabolism under toxicant stress. The possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress conditions. (Serebrov *et al.,* 2006) who stated that the *B. brongniartii* EPF had an effect on detoxification enzyme activity in *G. mellonella*. Fungal infection of insects increases Glutamic oxalic transaminase (GOT) in the hemolymph. *M. anisopliae* was susceptible hosts via direct penetration of the cuticle and potentially determining interaction and the spores were infective insect epicuticle (Fan *et al.*, 2013).

Table (5) : Changes in transaminase enzymes, ALT and AST activity enzyme in last instars larvae of *G. mellonella* treated with the three biocide products *B. bassiana, M. anisopliae* and *T. album* entomopathogenic fungi after five periods.

Treatments		Transaminase (µg pyruvate/g.b.wt./min. /days)									
		ALT					AST				
		1 day	3 day	5 day	7 day	10 day	1 day	3 day	5 day	7 day	10 day
B. bassian	SA	289.21a	116.56b	207.50a	152.90a	56.23d	45.82a	48.28a	89.73ab	153.90b	10.04c
	RA %	476.35	89.44	48.36	112.28	120.93	135.10	175.10	645.27	835.56	-59.99
M. anisopliae	SA	91.19a	73.64a	214.90b	273.50b	228.34a	42.79b	27.35c	21.10a	25.01a	65.15a
	RA %	81.73	19.68	53.66	279.70	797.17	119.55	55.84	75.25	52.04	159.56
T. album	SA	80.74c	101.77c	192.50c	130.19d	180.90b	12.89c	7.62b	10.57b	5.69c	52.55b
	RA %	60.91	65.39	37.64	80.74	610.90	-33.86	-56.58	-12.21	-65.41	109.36
Control		50.18b	61.53b	139.86c	72.03c	25.45c	19.49d	17.55d	12.04c	16.45d	25.10d
p		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$LSD_{0.05}$		5.78	2.73	4.68	4.68	7.89	11.06	8.14	13.62	11.07	5.97

P= Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately SA= Specific activity RA%= Relative activity % Increase or decrease than control = treated - control ÷ control ×100.

4. Phenol Oxidase:

Data in table (6) phenol oxidase of the treated last instar larvae of *G. mellonella*, the three tested fungi caused significant decreased in the activity of enzyme after five periods and reached to (52.38, 40.26, 11.23, 46.95 and 104.76 O. D. unit/min/ g.b.wt.) for *B. bassiana*, meanwhile in the case of *M. anisopliase* phenol oxidase enzyme gave (39.53, 24.19, 9.43, 42.31 and 91.67 O.D.unit/min/g.b.wt.). Also data cleared that, treated *G. mellonella* larvae with *T. album*, recorded the severely decreased in the activity of enzyme recorded (23.98, 7.89, 24.53, 12.25 and 25.93 O.D.unit/min/g.b.wt.) after five periods of treatment as compared with (29.51, 68.75, 29.89, 34.15 and 116.67 O.D.unit/min/g.b.wt.), respectively in control treatment.

The present results are accordance with some researchers such as, (González and Córdoba, 2012) who measured phenol oxidase which considers a key component in the immune system of insects and the main role of phenol oxidase in melanogenesis is converting phenols to quinones, which subsequently polymerize to form melanin. Also the results in agreeing with Elbanna *et al,*. (2012) who showed that, the insect enzymes Phenol oxidase activities were affected, fluctuated between increasing and decreasing by fungi infection.

Table (6)**:** Changes in activities of Phenol oxidase, Total protein and Total lipid enzymes in last instars larvae of *G. mellonella* treated with the three biocide products *B. bassiana , M. anisopliae* and *T. album* entomopathogenic fungi after five periods**.**

Treatments		Phenol oxidase (O.D. unit/ min/g. b. wt.)					Treatments	Total protein (µg/g. larvae)/days					Treatments	Total lipid (µg/g. larvae)/days				
		1 day	3 day	5day	7 day	10 day		1 day	3 day	5 day	7 day	10 day		1 day	3 day	5 day	7 day	10 day
B. bassian	SA	52.38a	40.26b	11.23c	46.95a	104.76a	SA	25.19c	2.79d	4.40b	7.59b	4.76c	SA	258.49b	321.50a	483.50a	366.50a	198.70a
	RA %	77.50	-41.44	-62.43	37.49	-10.20	C%	-68.05	-82.26	-65.24	-52.92	-67.22	C%	1.40	66.12	66.50	45.84	96.93
M.anisopliae	SA	39.53b	24.19c	9.43d	42.31a	91.67b	SA	3.79b	6.21b	3.01b	6.50b	10.80b	SA	376.70a	319.68ab	325.22ab	314.07b	194.54a
	RA %	33.96	-64.81	-68.45	23.90	-21.43	C%	-74.72	-60.51	-76.22	-59.68	-25.62	C%	47.77	65.18	12.06	24.98	92.8
T.album	SA	23.98c	7.89d	24.53b	12.25c	25.93c	SA	4.61b	4.68c	4.29b	1.67c	4.17c	SA	351.03a	245.37ab	507.90a	269.63b	180.50a
	RA %	-18.74	-88.52	-17.93	-64.13	-77.78	C%	-69.25	-70.25	-66.11	-89.64	-71.28	C%	37.70	26.78	74.99	7.29	78.89
Control		29.51c	68.75a	29.89a	34.15b	116.67a	SA	14.99a	15.73a	12.66a	16.12a	14.52a	SA	254.93b	193.53b	290.23b	251.30b	100.90b
P		0.000	0.000	0.000	0.000	0.000	P	0.000	0.000	0.000	0.000	0.000	p	0.000	0.010	0.010	0.000	0.000
$LSD_{0.05}$		11.21	10.96	3.39	9.52	15.86	$LSD_{0.05}$	2.04	1.49	2.92	1.78	3.68	$LSD_{0.05}$	84.44	114.31	115.22	88.57	29.13

P= Probability Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately SA= Specific activity RA%= Relative activity % Increase or decrease than control = treated - control ÷ control ×100. C= Change activity

5. Total Protein Enzyme:

Data in Table (6) revealed that, the changes in total protein on *G. mellonella* larval homogenate treated after five periods. Data showed that, *B. assiana*, *M. anisopliae* and *T. album* treatments at five periods caused highly significant decreased in total protein. In case of *B. bassiana* recorded (2.79, 4.40, 7.59 and 4.76 mg/g.b.wt.) after three, five, seven and ten days, respectively from treatment. Meanwhile the change of total protein content increased and gave (25.19 mg/g.b.wt.) after one day from treatment. While *M. anisopliae* gave a fluctuated decrease ranged between (3.01 to 10.80 mg/g.b.wt.) after five periods. Also, data cleared *T. album* fungi were the most treatment effect and caused highly a fluctuated decrease in total protein ranged between (1.67 to 4.68 mg/g.b.wt.) after five periods of treatment as compared with control.

6. Total Lipid Content:

Results cleared that, gradually significantly increased in total lipid up to ten days in the three treatments. The specific activity on total lipid on last instar larvae of *G. mellonella* larval homogenate after five periods. Data showed that, the three tested biocides treatments produced a highly significant increase, in total lipid content and recorded (483.5 mg/g.b.wt.) after five days from treatment of insect for *B. bassiana*, but in *M. anisopliae* gave (376.60 mg/g.b.wt.) after one day followed by (325.22 mg/g.b.wt.) after five days of treatment. While *T. alum* fungi were the most treatment effect *G. mellonella* larvae and caused highly increased in total lipid gave (507.90mg/g.b.wt.) after five days, while after one day the total lipid reached to (351.03 mg/g.b.wt.) as compared with control treatment Table (6).

Generally, results showed *B. bassiana*, *M. anisopliae* and *T. album* had a significant role in the phenol enzyme causing fluctuated significant decreased and increased. While the three tested biocides caused a highly significant decrease in total protein. On the contrary, in total lipid the tested fungi caused a significant increase in the incorporation of amino acids in protein. Many researchers stated that, the results agreed with (Serebrov *et al.*, 2006) who tested the effect of EPF on some biochemical aspects as detoxification enzyme activity in *G. mellonerlla* larvae and role of detoxification enzymes in development of insect resistance to fungi male *G. melonella* moths were more tolerant to *B. bassiana* in all treatments . (Pham and Schneider, 2008) reported that, the cuticular phenoloxidase (PO) is a melanizing enzyme at the wound site which limits infection; PO in hemolymph is responsible for melanotic immune responses. The PO immune responses occur immediately against invading microbes

in insects (Castillo et al., 2011) and (González and Córdoba, 2012) who showed that, PO is a key component in the immune system of insects and the main role of PO in melanogenesis is converting phenols to quinones, which subsequently polymerize to form melanin. Other researchers stated that, total proteins, carbohydrates and lipids contents were dramatically declined in all treatments (Elbanna et al., 2012). Some researchers measured total proteins, carbohydrates and lipids contents of *Agrotis ispelon* in the tested insects by Kleespies *et al*. (2013). The effect of *B. brongniartii* and its secondary metabolites on the detoxification enzymes of *D.tabulaeformis* larvae (Fan *et al.*, 2013). The tested *M. anisopliae* were increasing and decreasing enzymes Phenol oxidase activities, Total proteins, carbohydrates and lipids contents were also dramatically declined (Ortiz *et al.*, 2013). Penetration of the cuticle requires the release of fungal enzymes, including proteinases. Here EPF fungi can in turn sense the presence of insect-derived antifungal peptides and proteinase inhibitors *in G. melonella* treated with fungus *Metarhizium robertsii* (Vilcinskas and Virulence, 2018).

Conclusions:

The three tested biocides as natural product *B. bassiana, M. anisopliae* and *T. album* can be used for control *G. mellonella* larvae as a bio-control agent. The LC_{25} and LC_{50} for both biocides after 72 hours were determined. At LC_{25} and LC_{50} the toxicity effect of *T. album* fungi recorded high toxicity effect against *G. mellonella* and caused a high effect at relative potency as fold. On the other hand, a significant decrease in some biological aspects. *M. anisopliae* shortened the period of insect life while *B. bassiana* gave the highest percentage of pupal mortality and reduced weight of pupae, the tested biocides caused decreased pupation percentages, and except *B. bassiana* caused high pupation than control. While the *M. anisopliae* had significantly reduced the rate of adult emergence. But *T. album* reduced the female's longevity while *M. anisopliae* led to an increase in the malformation percentage resulting from larval treatment. Through these results, these vital products can be used in honey bee colonies to combat the *G. mellonella* as a biological control. Also, results showed that the three tested biocides had an effect on the larvae of the greater wax moth, where disturbance the activity of the amylase enzyme reduced after five periods of treatment compared to control. There was also a significant increase in protease enzyme activity compared to control. The activity of alanine aminotransferase (ALT) was highly significant increased. On the other hand, the aspartate aminotransferase enzyme (AST) showed gradually fluctuated effect by three tested biocides. While the three tested fungi caused a significant decrease in the activity of phenol oxidase enzyme and a highly significant decrease in total protein content, while the levels of changes in total lipid content recorded a significant increase in all the treatments compared to control.

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ARABIC SUMMERY

تقييم فعالية بعض الفطريات الممرضة للحشرات على يرقات فراشة الشمع الكبرى جاليريا ميلونيلا (حرشفيه الأجنحه: جاليريدى)

ابراهيم شكري [1] فرج عبد الحليم[1] كريمه خاطر [1]سهير فيصل[2] وهند محمد عبد المنعم[2]

1-علم الحشرات--كليه العلوم -جامعة الزقازيق
2-معهد بحوث وقاية النباتات-الدقي- جيزه

استهدفت الدراسة الحالية لمعرفة تأثيرثلاثة فطريات ممرضة للحشرات للاختبار ضد يرقات فراشات الشمع جاليريا ميلونيللا بمعاملتها بثلاثه فطريات ممرضة حشرية، بيوفاريا باسيانا (بيوفار)، تريكودرما البيوم (بيوزيد) و ميتاريزيم انيزوبليا (بيورانزا). أظهرت النتائج عند قيم التركيز الربع مميت للفطريات الثلاثة تريكودرما ألبوم ، ميتاريزيم انيزوبليا و بيوفاريا باسيانا ضد يرقات فراشة الشمع كانت 14.08، 44.20 و57.82 جزء فى المليون ، على التوالي. بينما عند التركيز النصفي المميت كانت قيم السميه 104.03 ،252.55 و389.05 جزء فى المليون ،على التوالي لنفس المركبات الحيويه. وجد ان تريكودرما ألبوم اكثر المنتجات سميه لمقاومة يرقات فراشة الشمع. بينما بيوفاريا باسيانا كان اقلهم سميه. وتم قياس ميل خط السمية للمركبات المختبرة (0.81 ، 0.89 و 0.78). وكان دليل السمية للمركبات المختبره 24.35 & 26.74 & 31.85 & 41.18 على التوالي. على الجانب الاخر كانت الكفاءة النسبية للمركبات الثلاثة المختبره عند التركيز الربع والنصف مميت 1.31 & 1.54 ضعف لميتاريزيم انيزوبيليا ويليه 4.11 & 3.74 ضعف لتريكوديرالبيوم ما مقارنة بـ 1.00 ضعف في حالة بيوفاريا باسيانا. أظهرت النتائج أن نسبة الموت التراكمي لليرقات المعامله بالمنتجات الثلاثة يمكن ترتيبها وفقاً للمركبات الأكثر كفاءة كالتالي 47.61 ، 41.52 و 39.67٪ على التوالي لتريكوديرالبيوم، ميتاريزيم انيزوبيليا و بيوفاريا باسيانا بالمقارنة مع 0.00٪ في اليرقات الغير معامله. أيضاً، أشارت النتائج إلى أن المبيدات الحيوية التي تم اختبارها على يرقات جاليريا ميلونيلا أثرت معنويا على بعض النواحى البيولوجية. وجد ان بيوفاريا باسيانا من اكثر المركبات تاثيرا على مدة الطور اليرقى والعذري، معدل موت العذاري، وزن العذاري والنسبة الجنسيه. في حين ، ميتاريزيم انيزوبيليا خفض فترة حياه الذكور ومعدل التشوه للحشرات الكامله سجلت أعلى نسبة تشوه 27.35٪ مقارنة بالكنترول. بينما تريكوديرما البوم كان الاكثر سمية على معدل الموت التراكمى لليرقات ، نسبة التعذير ،مدة طور العذراء ايضا خفض معنويا مدة طور الاناث الفراشات. أكدت نتائج تلك الدراسه ان المعاملة بالمنتجات الفطريه المختبره تؤثر على فسيولوجية يرقات العمر الاخير لفراشة الشمع مما يؤدي إلى خلل فى نسب كل من المحتوى الكلى للدهون والبروتين في كل معاملة من المعاملات ونشاط بعض الأنزيمات. حيث خفضت نشاط انزيم الاميليز مقارنة باليرقات الغير معاملة. ولوحظ ان الثلاث مركبات فطرية تسببت في زيادة معنوية لنشاط انزيم البروتييز عن الكنترول. بينما سجل خلل فى نشاط الإنزيمات الناقلة للأمينAST وALT مقارنة بالكنترول. ولوحظ خفض معنوى فى نشاط انزيم الفينول أوكسيديزلكل الفطريات المختبرة وكذلك تقل أيضاً نسبة المحتوى الكلي للبروتين للمعاملات عن الكنترول وسجلت النتائج زيادة معنوية فى المحتوى الكلى للدهون في كل المعاملات مقارنة بالكنترول.

ومن هنا يتضح أن لهذه المنتجات الحيوية التي تم اختبارها تأثيراً فعالاً ضد مكافحة يرقات فراشة الشمع جاليريا ميلونيلا وبالتالي يمكن استخدامها في مستعمرات نحل العسل كإستراتيجية فى برامج المكافحه الحيويه و كبديل آمن للمبيدات والمركبات الأخرى ذات التأثير الجانبي الضار على كل من الإنسان والبيئة