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Study of Infection Impact by Entamopathogenic Fungi on some Biochemical Contents in Haemolymph of Cotton Leaf Worm, *Spodoptera littoralis* (Boisduval)

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ABSTRACT

Carbohydrates, lipids and proteins contents were evaluated in 5th larval instar haemolymph of *Spodoptera littoralis* (Boisd.) that treated with four isolates of entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae, Paecilomyces lilacinus* and *Lecanicillium antillanum* at 1st, 2nd, 3rd and 4th days. Generally, data in present work cleared that, there was significant decrease in haemolymph carbohydrates of larvae injected with the entomopathogenic fungi in the three time intervals (1st, 2ndand 3rd) under investigation compared to control. The concentration level of protein content of larvae injected with the fungi significantly increased as compared with control insects in (1st) day, then there is no significant change in the content after 2nd and 3rd day except increasing was noticed with the two fungi (*B. bassiana* and *L. antillanum*).There were significant increases of carbohydrates and protein contents observed in the last day (4th day) due to effect of fungal infection. Significant increases of lipid content was noticed in the 5thinstar cotton leaf worm larvae inoculated with the four tested fungi from 1stto 4th day. The overall results indicated that total *S. littoralis* larvae proteins, carbohydrates and lipids contents vary significantly during infection of the tested fungi at the four time intervals (1st, 2nd 3rdand4th days) and this tend to dramatically changes in the composition of haemolymph, thus abnormal growth and development of insect caused.

Keywords: Carbohydrates, lipids and proteins contents, Fungi, *Spodoptera littoralis*.

INTRODUCTION

The action of entomopathogenic fungi caused mortality in tested pests; the agents are safe and worldwide interest of improvement and using for insect biological control. The infect by entomopathogenic fungi caused it reaches to hemocoel, and blastospores produces are final pathogenic stage of infection host (Vincent *et al*., 2007). The insects Haemolymph is a medium for some processes of physiological, such as, intermediary of metabolism and responses of immune. When enters the invader to insects hemocoel, the hemocytes are engaged to remove non-self-target by phagocytosis, encapsulation, nodule form, antimicrobial peptides synthesis and reaction of metabolites (Beckage, 2008). The haemolymph of Insects, Consists of complex mixture of lipids, proteins, acids, carbohydrates, hormones and degradation product of them, is primary response for nutrients supply, metabolic transferring wastes to normal maintain of development and growth. Only haemolymph is a extracellular fluid of insects with various functions and products reservoir required for physiological activities of insect body, the composition changes of haemolymph reflect biochemical and physiological transformations in tissues of insect (Pawar and Ramakrishnan1977).

The bio-molecules (carbohydrates, proteins and lipids) play a principle role in the development and morphogenesis in the intermediary metabolic pathways of insects. Intermediary metabolism consists of various pathways in which ingested and stored nutrients such as carbohydrates, lipids and proteins are processed to produce energy via their degradation or synthesis (Nation, 2008).

Spodoptera littoralis (Boisduval), the Egyptian cotton leaf worm (Lepidoptera: Noctuidae) is the serious polyphagous insect, it an economically important pest of many agricultural crops. It was reported that it can attack 112 plant species belonging to 44 families (Mallikarjuna *et al.,* 2004). Many entomopathogenic fungi were found to be efficient biological agents to control this pest. Although extensive studies have been conducted on the process of cuticle penetration by entomopathogenic fungi effect of entomopatognic fungi on haemolymph components still under-explored. Pathogenicity of entomopathogenic fungi may occur via overcoming on immune responses and discrepancies of intermediary metabolisms. The progress of infection by a pathogen in the host tissue can be monitored by studying the degree of variation in metabolic constituents (Rajitha and Savithri 2014).This may be caused an impact on the role of enzymes in insect resistance to entomopathogenic fungi for this, it was necessary to continue studies to evaluate the influence of four isolates of entomopathogenic fungi on some biochemical changes in haemolymph components of *S. littoralis.*

MATERIALS AND METHODS

Insect used

Newly moulted 5thinstar larvae of *Spodoptera. littoralis* Bois. were obtained from culture reared for several generations on castor leaves under laboratory conditions (27.0 ± 1.0ºC and 70.0 ± 5.0%RH).

Fungal cultures

Blastospores cultures of entomopathogenicfungi, *Beauveria bassiana*, *Metarhizium anisopliae, Paecilomyces lilacinus* and *Lecanicillium antillanum* produced by inoculating conidia into Sabouraud Dextrose broth. This medium was prepared and adjusted at pH (5.5-6.0). The culture was inoculated at 25±10^{o}C on shaker for 5 days. The culture was filtered through sterilized glass wool to remove mycelia. Blastospores were collected from filtrates by centrifugation and washed twice in a sterile solution of 0.85% saline. Fungal cells were counted on a hemacytometer and diluted in sterile saline solution to a concentration of 1x10^{7} blastospores⁄ml for entomopathogenic fungi . Five µl of the concentration were injected into the hemocoeles of fifth instar of *S. littoralis* larvae.The control larvae injected with five µl of sterile 0.85% saline solution. While the treated and control larvae were placed in sterile batches containing cleaned castor bean leaves, and then incubated at 25±10^{o}C and 75±5% RH. The injected larvae were surface sterilized with 70% ethanol and rinsed twice with sterile distilled water (Gabarty *et al*.,2013).

Collection of haemolymph

After 1, 2, 3 and 4 days of the induction with fungal pathogen, haemolymph was collected from 5th instar *S. littoralis* larvae into eppendorf tubes containing a few milligrams of phenylthiourea to prevent the inhibitory effect of tyrosinase (Abou El-Ghar *et al*., 1996) by clipping third pair of abdominal legs of larvae.

Biochemical assays:

Estimation of the total haemolymph proteins.

Proteins content of *S. littoralis* haemolymph was determined spectro-photometrically according to Bradford (1976) with bovine serum albumin (BSA) as the standard protein solution. The method depends on the protein forms a violet complex with cupric ions in alkaline medium. The absorbance at 595 nm was measured after 2 minutes and before 1 hour, against a blank. Proteins content was expressed as mg/ml haemolymph.

Estimation of the total haemolymph carbohydrates

Total carbohydrate content in haemolymph was quantitatively determined by phenol-sulfuric acid reaction according to Dubois *et al*., (1956). The absorbance of yellow-orange color was measured at 490 nm against blank. Total carbohydrates content were calculated and expressed as mg /ml haemolymph.

Estimation of the total haemolymph lipids

Total lipids were determined according to Knight *et al.,* (1972). The developing rose color was measured at 525 nm against a blank. Lipids content was expressed as mg /ml haemolymph.

Statistical analysis:

Data of all experiments were evaluated statistically using ANOVA and means were compared using Duncan's Multiple Range Test at ($P<0.05$). All statistical analyses were done using the software package Costat.

RESULTS AND DISCUSSION

This study gives basic information about how the insect haemolymph nutrients could be affected due to injection with high virulent of entomopathogenic fungi. The difference in the total protein, carbohydrates and lipids contents in haemolymph from1st to 4th days during 5th instar development in *Spodoptera*. *littoralis* Bois. infected with four fungi were studied.

1- Effect of fungi on haemolymph protein content.

The investigation of biochemical changes in haemolymph protein content level (mg/ml) of the 5th instar larvae of *S. littoralis* injected by $1x10^7$ blastospores/ml of four entomopathogenic fungi, *B. bassiana*, *M. anisopliae, P. lilacinus* and *L. antillanum* after 1, 2, 3 and 4 days, demonstrated in (Table 1).

Results showed that the four fungi caused increase in haemolymph protein content of the treated *S. littoralis* 5th instar at 1st day, the highest increasing were recorded with *L. antillanum* (25.00 mg/ml) then *M. anisopliae* (21.80 mg/ml), *B. bassiana* (20.00 mg/ml) and finally *P. lilacinus* (19.8 mg/ml), compared to control (18.2 mg/ml). The increasing of protein content continued at the 2ndday with *B. bassiana* (39.60 mg/ml), but, there were decreases in protein content with *P. lilacinus* (31.00 mg/ml), *M. anisopliae* (30.40 mg/ml) and *L. antillanum* (29.60 mg/ml) compared to control (32.00 mg/ml) at the same day. At the 3rd day, only protein content of *M. anisopliae* (46.00mg/ml) and *B. bassiana* (42.60mg/ml) were higher than control (37.80mg/ml) while, the other three fungi showed decreased protein content. There was increase in protein content at the last day (4th day) with all tested fungi except *M. anisopliae* (23.80mg/ml). The other fungi achieved increasing protein content as follow *P. lilacinus* (33.80mg/ml), *B. bassiana* (31.00mg/ml) and *L. antillanum* (30.80mg/ml) compared to control (25.60mg/ml).The results of the study shows a series of variations in total protein content in 5th instar *S. littoralis* larvae infected with four fungal pathogens.

Highly significant protein levels (increase)were noticed in the larvae of 5th instar *S. littoralis* from 1stday to 3th day with the fungal pathogen of *M. anisopliae* (21.80 to 46.00mg/ml) and *B. bassiana* (20.00 to 42.60mg/ml),whereas, significant variations (decrease) in total protein content were recorded with *P. lilacinus* (19.80 to 33.80mg/ml), *L. antillanum* (25.00 to 31.00mg/ml) from 1stday to 3th day compared to the control variations (18.20 to 37.80mg/ml) at the same periods. But, there were decreasing in protein levels were noticed from 3thto the last day(4th day) of inoculated *S. littoralis* with the four fungi and control (Table 1). Generally, in the control and the four fungi, protein level increased day by day from 1st to 3th day, but decrease with the last day. In general, the protein content level of the 5th instar larvae of *S. littoralis* in the haemolymphwas found maximum on third day, but decreased at the last day 4th day, due to fungi infection. The overall results showed, there are increasing in the haemolymph protein content with increasing of time.

Proteins are fundamental components of all living cells and include many substances, such as enzymes, hormones, and antibodies that are necessary for the proper functioning of an organism. The digestive activities are high during the early part of 5th instars development, so protein content from first day to third day was increased, which results in increased accumulation of proteins that are then transported to other tissues through the haemolymph for further physiological activities (Horie *et al.,* 1982).

Table 1. Effect of five entomopathogenic fungi on proteins content (mg/ml) in haemolymph of 5thinstar larvae of *Spodoptera littoralis.*

Treatments	Protein Mean ± S.D (mg/ml)			
	Time (days)			
	1st	2nd	3rd	4th
Control	18.20±0.74d	32.00±0.47c	37.80±1.06c	25.60±0.33d
M. anisopliae	21.80±0.30b	30.40±0.45d	46.00±0.25a	23.80±0.95c
P.lilacinus	19.80±0.35c	31.00±0.25d	33.80±0.60d	33.80±0.40a
L. antillanum	25.00±0.25a	29.60±0.35e	31.00±0.20f	30.80±0.40b
B. bassiana	20.00±0.21c	39.60±0.45a	42.60±0.35b	31.00±0.55b
F value	43.94***	239.18***	598.06***	157.99***
LSD0.05	1.09	0.744	0.744	0.744

Nirupama, (2015) showed that, the inoculated batches of protein content level of silkworm, *Bombyx mori* was maximum on third day in the haemolymph and decreased gradually towards the end of 4thand 5thdays due to the fungi infection. Also our results were confirmed by Vidhya, *et al* (2016) they stated that, infection of the army worm *Spodoptera litura* (Fabricius) by three fungal pathogens *B. bassiana, M.anisopliae*and *Verticillum lecanii* showed significant increase of protein content at 48hrs and significantly decreased at the 96 hrs, as compared to control.In contrast, Padma and Ramani (2015)found that the larvae of mulberry silkworm, *B. mori* fed with *B. bassiana* treated leaves at 1stto 6thday of the fifth instar recorded decrease in protein content compared to control. Also, Nada, (2015) reported that, the total of protein level of adult *Nezara virdula* treated with *M. anisopliae* were significantly decreased than the treatment with *B. bassiana*, after 24, 48 and 72 hrs.

2- Effect of fungi on haemolymph carbo hydrates content.

Total carbohydrates content divergence in haemolymph of 5th instars *S. littoralis* was presented in (Table 2).

A significant increase was recorded in the 1st day of fungal infection with *B. bassiana*, (13.2mg/ml) and *L. antillanum* (12.3 mg/ml) compared to control (10.30 mg/ml), but, there were noticed a significant decrease was recorded with *M. anisopliae* (6.80 mg/ml) and *P. lilacinus* (8.80 mg/ml) at the same day. Then fungi at 2nd and 3rd day, *M. anisopliae* (10.40 and 12.90mg/ml), *P. lilacinus*(12.60and13.90 mg/ml), *L. antillanum* (11.00 and10.50mg/ml) and *B. bassiana*(10.30 and 8.10 mg/ml) respectively, were caused significant variation (reduction) in carbohydrates content ompared to their control (13.30 and 14.70 mg/ml) respectively. There was increase in all fungi again noticed at the last day (4th day).

Results cleared that only three fungi caused increase in the haemolymph carbohydrates content of the infected *S. littoralis* 5th instar from 1st to 3rd day, these increasing ranged from 6.80 to 12.90mg/ml recorded with *M. anisopliae*, and 8.80 to 13.90 mg/ml recorded with *P. lilacinus*. The other two entemopathogenic fungi *L. antillanum* and *B. bassiana* decreased carbohydrates content from 12.3 to 10.50 mg/ml and from 13.20 to 8.10 mg/ml respectively at the same time period. There were decreasing in carbohydrates levels noticed on the last day (4thday) of inoculated *S. littoralis* except *L. antillanum* and *B. bassiana* recorded increasing in carbohydrates levels compared to control. On other hand, haemolymph carbohydrates content of control increased from 10.30 at 1st to 14.7mg/ml at 3rd day, then significant reduction occurred at 4th day (9.20mg/ml) (Table 2). In general, there are increasing in the haemolymph carbohydrates content with increasing of time from 1st to 4th day except there are decreasing recorded with *P. lilacinus* and *M. anisopliae*.

Table 2. Effect of five fungal pathogens on carbohydrates contents (mg/ml) in haemolymph of 5th instar larvae of *Spodoptera littoralis.*

Treatments	Protein Mean ± S.D (mg/ml)			
	Time (days)			
	1st	2nd	3rd	4th
Control	10.30±0.30c	13.30±0.20a	14.70±0.35a	9.20±0.35f
M. anisopliae	6.80±0.25e	10.40±0.50e	12.93±0.45d	12.43±0.25c
P.lilacinus	8.80±0.60d	12.60±0.15c	13.90±0.40c	9.80±0.70e
L. antillanum	12.30±0.30b	11.00±0.25d	10.50±0.50e	14.30±0.40b
B. bassiana	13.20±0.35a	10.30±0.45e	8.10±0.06f	17.70±0.85a
F value	93.69***	550.45***	1966.40***	3382.6***
LSD0.05	0.822	0.1779	0.1779	0.1677

These results agree with Nada, (2015) mentioned the total protein level in adult of *N. virdula* when treated with *M. anisopliae* gave significantly decreased than others treated with *B. bassiana* during time 24, 48 and 72 hrs. Also, Nirupama, (2015) showed that, total carbohydrates content level in the haemolymph of *B. mori* 5thinstar showed normal in the 1stday and decreased gradually towards the end of 5thday in inoculated batches. On other hand, the results of Meshrif *et al*., (2010) indicated that, there was initial significant increase in plasma carbohydrates of *S. littoralis* 5th instar larvae when injected by fungi (*Nomuraea rileyi* and *B. bassiana*) and in the subsequent time intervals, no significant changes between the control and the infected insects were observedunder investigation.

Since, carbohydrates providing the major of energy sources production for growth cells and development, serve as structural blocks of cells building and intermediates of metabolic (Lee *et al*., 2002), so, the decreasing at 1st, 2nd and 3rd day in the total carbohydrates of *S. littoralis* infected may be considered as energy reserves required for defense reactions and other vital processes. Also, the reduction significant of content carbohydrate noticed by 1st, 2nd and 3rd day of inoculated larvae compared with control, may be due to carbohydrate utilization ceased by food intake and pathogen. Levels of carbohydrates were decreased in haemolymph and attributed to carbohydrate excessive utilization required to give energy of infection. Sarma *et al.,* (1994). Reported that, the steady decreases in carbohydrate contents as infection of nuclear polyhedrosis virus (NPV) progresses up to 5th day of 5th instar for silkworm *B. mori* to carbohydrates utilization as a source of energy required for biosynthesis of viral constituents. Mallikarjuna *et al.,* (2002) mentioned that, the total content of carbohydrates were decreased as a developed disease and reasonable to mentioned that, carbohydrates used as a source of energy required for fungus development and growth. The haemolymph of insect has a large pool of sugars and it is reasonable to assume that the depletion of glycogen in the tissues target may be result of utilization as an energy source required for metabolism increased in host ceased by infection Kobayashi and Kawase, 1981.

3- Effect of fungi on haemolymphlipids content.

Data given in Table (3) indicated that the changes in the total content of lipids in larvae of *S. Littoralis* treated with the tested four entomopathogenic fungi at the 1st , 2nd , 3rd and 4th days.

The results showed that a significant change in the total content of lipids in the larvae treated with the entomopathogenic fungi tested as compared with untreated the larvae (control). *P. lilacinus* (5.14 mg/ml) and *L. antillanum* (4.5 mg/ml) were recorded the highest increase in lipid content, while the lowest value was recorded with *B. bassiana* (2.7mg/ml) compared to control (2.5 mg/ml) in1stday larvae of *S. littoralis*. All tested fungi increase in lipid content at 2nd day and the highest increasing values were achieved by *B. bassiana* (5.12 mg/ml), *L. antillanum* (4.08 mg/ml) compare with control (2.48 mg/ml). Also, there was significant increasing in lipid content of larvae of *S. littoralis* at 3rd day treated with *B. bassiana* (4.84 mg/ml), *L. antillanum* (4.82 mg/ml) and *P. lilacinus* (4.76 mg/ml) compared to control (3.32 mg/ml).On the other hand, there was reduction in lipids content with *M. anisopliae* (2.40 mg/ml). The four tested fungi showed increasing in lipid content of the treated larvae at 4th day and the significant increasing recorded with *B. bassiana* (7.62 mg/ml) followed by *L. antillanum* (5.30mg/ml) compared to control (3.40 mg/ml)

Lipid content level was increased when larvae of *S. Littoralis* treated with the tested fungi and ranged from 2.70 to 7.62 mg/ml (*B. bassiana*), 2.60 to 4.60mg/ml (*M. anisopliae*), 4.50 to 5.30 mg/ml (*L. antillanum*) from 1stto 4th day.While, *P. lilacinus* caused decreasing in lipid content level from 5.14 to 3.88mg/ml at the same time. On the other hand, lipids content of control approximately not changed (Table 3).Therefore treatment of larvae of *S. littoralis* with the tested fungi tend increase of lipid content level with time increasing.

Table 3. Effect of five fungal pathogens on lipids contents (mg/ml) in haemolymph of 5th instar larvae of *Spodopteralittoralis*

Treatments	Protein Mean ± S.D (mg/ml)			
	Time (days)			
	1st	2nd	3rd	4th
Control	2.50±0.40e	2.48±0.22f	3.32±0.11c	3.40±0.33f
M. anisopliae	2.60±0.06de	2.70±0.17e	2.40±0.15d	4.06±0.06d
P. lilacinus	5.14±0.20a	3.56±0.09c	4.76±0.40a	3.88±0.04e
L. antillanum	4.50±0.32b	4.08±0.04b	4.82±0.10a	5.30±0.29b
B. bassiana	2.70±0.08d	5.12±0.02a	4.84±0.25a	7.62±0.17a
F value	446.95***	1665.49***	1713.56***	2049.52***
LSD0.05	0.163	0.074	0.074	0.104

Our results are agreement with Nirupama, (2015) found that, lipid concentration level in the haemolymph of silkworm, *B. mori* (5th instar) was high at the initial three days and simultaneously decreased their level at the end of 5thday in inoculated batches, while, but, the control gradually increased. The results of Gabarty, (2011) showed a significant increase in the total content of lipids in the greasy cut-worm *Agrotis ipsilon* (Huf.) larvae treated with the two pathogenic fungi, *B. bassiana* and *M. anisopliae*as compared with control. In contrast to the present study, the obtained data of Nada, (2015) showed that total lipids decreased significantly when adults of *N. virdula* treated with *M. anisopliae* and their no significant differences during 24, 48, 72 hours.

Lipids are important source of energy reserves compared to carbohydrates. Lipids can supply as much as eight time more energy per unit weight (Beenakkers *et al.*, 1985 and Ali, 2011). The important components of cuticle are Lipids, which help in acylation of glucose-6-phosphate during chitin synthesis (Wyatt, 1967). Lipids are fundamental for structural components of cells and serve as a source of metabolic energy. The lipid can be mobilized rapidly during starvation, embryogenesis, oogenesis and moulting in the fat body so considered as an energy reserve and is used to sustain continuous muscular activity (Gilbert and Chino, 1974).

The biochemical parameters such as, proteins, carbohydrates, lipids, nucleic acids *etc.*, vary significantly during the life cycle of all living organisms. Metabolic changes play an important role in understanding the interaction between the host and pathogen as a part of a survival strategy. Several biochemical and physiological alterations caused in insect tissues owing to pathogenic infections (Martignoni, 1964; Shigematsu and Noguchi, 1969).The infection progress by a pathogen in the host tissue can be monitored by studying the degree of variation in metabolic constituents (Rajitha and Savithri 2014).

Generally, in the present study, biochemical changes occurred in the total protein, carbohydrate and lipid contents in the haemolymph during the course of fungi infection. The present findings support that infection with entomopathogenic fungi had different effects on the 5th instar of *S. littoralis*, particularly as they changed haemolymph nutrients contents (e.g. Carbohydrates, lipids and proteins).The increase and decrease in the proteins, carbohydrates and lipids contents of heamolymph from the 1st day up to 4thday after inoculated due to infection by pathogen of fungi have important role in *S. littoralis* growth and development. Infection of *S. littoralis* with fungi tend to its stimulated their protein, carbohydrate and lipid utilization in order to meet requirements of toxic stress. Under fungi infection there are correlation between the changes in the concentration of these bio-molecules and the degree of their absorption, interconversion and utilization and the high toxicity of the *S. littoralis* larvae.

The biochemical results showed these major changes in protein, carbohydrate and lipid content level in infected causes for high toxicity of the *S. littoralis* larvae showing significant variation in their contents. The results indicated fungi infection caused physiological and biochemical changes in the cotton leaf worm. Metabolic changes play an important role in understanding the interaction between the host and pathogen as a part of a survival strategy. Metabolite depletion by the entomopathogenic fungi could cause physiological imbalances in the host that lead to changes in enzyme activates and a reduction in haemolymph protein, carbohydrates and lipid contents.

CONCLUSION

In conclusion, the results of this study clearly indicated that, fungi caused a sharp disturbance in the protein, carbohydrate and lipids contents under highly toxic effect by fungus which produced a toxins impact on the leaf-worm *S. littoralis*, consequently these biocontrol agents can used as a promising environmentally friendly alternatives of the synthetic chemical insecticides against this destructive pest *S. littoralis*. However, more research is needed to investigate the *in vivo* and *in vitro* determination of virulence factors of entomopathogenic fungi.

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تأثير العدوى الفطرية الممرضة للحشرات على بعض المحتويات البيوكيميائية في هيموليمف حشرة دودة ورق القطن

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من خلال الدراسة تم تقييم وتقدير محتويات الكربوهيدرات والدهون والبروتينات الكلية في هيموليمف يرقات العمر الخامس لدودة ورق القطن التي عوملت بأربعة عزلات من الفطريات الممرضة للحشرات : *Beauveria bassiana*; *Metarhizium anisopliae*; *Paecilomyces lilacinus* and *Lecanicillium antillanum* . خلال أربعة فترات زمنية (اليوم الأول والثاني والثالث والرابع). عامة أوضحت النتائج أن هناك انخفاض معنوي في كربوهيدرات هيموليمف اليرقات المعاملة بالفطريات في الفترات الزمنية الثلاث (الأولى والثانية والثالثة) مقارنة بالكنترول. لوحظ زيادة في مستوى تركيز البروتين في اليرقات المعاملة بالفطريات في اليوم الأول بشكل كبير مقارنة مع حشرات الكنترول ، بينما لم يكن هناك تغير كبير في محتوى البروتين بعد اليوم الثاني والثالث ، إلا أنه لوحظ زيادة مع نوعين من الفطريات وهما *B. bassiana* and *L. antillanum* وقد وجدت زيادة معنوية في محتوى الكربوهيدرات والبروتين في اليوم الأخير (اليوم الرابع) بسبب تأثير العدوى الفطرية. ولوحظت زيادات كبيرة في محتوى الليبدات في يرقات دودة ورق القطن التي تم معاملتها بالفطريات الأربعة من اليوم الأول إلى اليوم الرابع . وأشارت النتائج الإجمالية للبحث إلى حدوث اختلال في محتوى وتركيز البروتينات والكربوهيدرات و الليبدات الكلية في هيموليمف الحشرة بشكل كبير خلال المعاملة بالفطريات المختبرة في الفترات الزمنية الأربعة (اليوم الأول والثاني والثالث والرابع) ، وهذا أدي إلى تغيرات كبيرة في تكوين وتركيب محتوي هيموليمف الحشرة وبالتالي حدوث نمو وتطور غير طبيعي للحشرات الناتجة .