EFFECT OF DISINFECTANTSON SOMEBIOCHEMICAL CHANGES IN THE SILKWORM, *BOMBYX MORI* L. INFECTED WITH *BEAUVERIA BASSIANA* (BALS) VUILL

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Abstract

Influence of five bed disinfectants known asLC (botanical mixture of *Lawsonia inermis* and *Curcuma longa*) Ankush(mixture of botanical and chemical), Amruth (botanical), FL (chemical mixture of formaldehyde and lime) and Vijatha (chemical) on some biochemical changes in the silkworms; *Bombyx mori* L.infected with *Beauveria bassiana* (Bals) Vuill was evaluated in the present study. The results showed that, infection of *B. mori* with *B. bassiana* reduced the total proteins, carbohydrates and lipids contents in haemolymph compared to the normal control and decreased the activity of the three carbohydrates hydrolyzing enzymes (Trehalase, amylase and invertase). Application of the five disinfectants to the infected silkworm, *B. mori* increased total proteins, carbohydrates and lipids contents as compared to the infected control (the inoculated control). All disinfectants were decreased the activity of the three carbohydrates hydrolyzing enzymes compared to the inoculated control except the disinfectant LC which increased trehalase enzyme activity and the disinfectant FL increased the amylase and invertase activities. According to these results, it could be concluded that, the use of the disinfectants caused elevation the total proteins, carbohydrates and lipids contents and this elevation support the defense of *B. mori* against fungi and reducing the disease development.

Keywords: Total proteins, Total carbohydrates, Total lipids, Silkworm, *Bombyx mori* L., Disinfectants.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L.is of great economic importance as a foreign exchange earner for many silk producing countries of the world (Krishnaswami *et al.,* 1992). The major diseases affecting the silkworm are protozoan, fungal, bacterial and viral diseases which are causing about 27-35% crop loss (Sudhakara Rao *et al.,* 2011). Both the pathogen and the host cell lose their individual identity and form a new unit with distinct physiological and characteristics. 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).The effect of the fungal infection on the metabolic adaptations may correlate with its effect on the general health and the rearing performance of the silkworm larvae (Rajitha and

Savithri, 2013). Insect haemolymph, a complex mixture of proteins, lipids, carbohydrates, nucleic acids, hormones and their degradation product, is primarily responsible for supplying nutrients, transferring metabolic wastes to maintain normal growth and development, thus changes in the composition of haemolymph reflect the biochemical transformations taking place in the insect tissues (Pawar andRamakrishnan, 1977).

The systemic fungicides (chemicals or botanical) prevent the proliferation of fungi in the haemolymph or preventing the growth of the fungi in the body cavity through enhancing the hemocyte mediated defense response (Mallikarjuna *et al.,*2002). Disinfection and hygiene play an important role in the elimination of the pathogens and prevention of diseases during silkworm, *B. mori* rearing. The bed disinfectants are approved for four different purposes: disease treatment, disease prevention, disease control and for health maintenance or growth promotion (Subramanian *et al*., 2009).

Based on the study of Taha *et al*. (2016), two disinfectants known as FL (Chemical mixture formaldehyde and lime) and LC (Botanical mixture of *Lawsonia inermis* and *Curcuma longa*), from the five tested bed disinfectants against the pathogenic fungi *B. bassiana* tend to increase cocooning percentage comparing with inoculated control. For this, it was necessary to continue studies to evaluate the influence of these five bed disinfectants LC (botanical mixture of *Lawsonia inermis* and *Curcuma longa*) Ankush (mixture of botanical and chemical), Amruth (botanical), FL (mixture of formaldehyde and lime) and Vijatha (chemical)on some biochemical changes in the haemolymph of silkworm larvae *B. mori* during the infection of *B.Bassiana*.

MATERIALS AND METHODS

Rearing of silkworm *B.mori*

The silkworm eggs of commercial Bulgarian hybrid were used. The larvae were reared according to Krishnaswami (1978). Kokuzo-27 mulberry variety leaves were fedto silkworms four times a day as recommended.

Fungal isolation and preparation:

The fungus was isolated from silkworm larva died from white muscardine using Sabouraud Dextrose Agar medium according to Meyling and Eilenberg (2006). Culture of *B. bassiana* was maintained on Potato Dextrose Agar slants and was sub-cultured in Petri dishes**.**The conidial concentration (1×10^7conidia /ml) was estimated using Neubar hemocytometer under a Leitz DialuX20 EB microscope (400x magnifications) according to Yeo *et al*. (2003).

Experimental bed disinfectants:

1- LC : *Lawsonia inermis* and *Curcuma longa* 1:1 (botanical)

2- Ankush (mix botanical and chemical)

3- Amruth (botanical)

4- FL: Formaldehyde and lime powder 0.25 : 0.75 (chemical)

5- Vijatha (chemical)

Infection of silkworm, *B. mori* larva with *B. bassiana*:

The newly exuviated fifth instar larvae were separated into seven groups with 30 larvae/group. Each group represents a treatment with five replicates. The insect was immersed in the conidial solution for 10s. One group immersed in distilled water only (Normal control), another group immersed in fungal spores solution only (infected or inoculated control). The other five groups were immersed in fungal spores solution then treated with the five tested bed disinfectants by dusting every morning over the larvae half an hour prior to feeding 5g/sq.ft bed area (Chavan *et al*., 2013).

Biochemical assays:

After 144 hrs. of fungal pathogen induction, haemolymph was collected from fifth instar larvae into eppendrof tubes with a pinch of thiourea.

Determination of total proteins, carbohydrates and lipids

Proteins content of silkworm haemolymph was estimated spectro-photometrically by the method of Bradford (1976). 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.

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

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

Carbohydrates hydrolyzing enzymes assays

The activity of carbohydrates hydrolyzing enzymes (Trehalase, amylase, and invertase) was determined according to method of Birk *et al.* (1962). The trehalase and invertase reaction mixtures consisted of 0.2 ml 3% trehalose (trehalase substrate) and 0.2 ml 4% sucrose (invertase substrate), 0.2 ml (0.2 M) acetate buffer (pH 5.4) and 100µl of haemolymph. The amylase reaction mixture consisted of 0.2 ml 2% starch (substrate), 0.2 ml (0.2 M*)* phosphate buffer (pH 6) and 100 µl of haemolymph. All test tubes were incubated at 37°C for exactly 60 minutes, and then 0.8 ml of 3,5 dinitrosalicylic acid reagent was added. The reaction mixture was heated

for 5 minutes at 100°C in a boiling water bath then immediate cooling in an ice bath. The optical density (OD) was measured at 550 nm using spectrophotometer.

Statistical analysis:

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

RESULTS AND DISCUSSION

Effect of disinfectants on the total proteins, carbohydrates and lipids contents.

Total proteins content:

The data in Table (1) showed that, the haemolymph proteins content in normal control larvae decreased from 58.48 mg/ml to 38.49mg/ml (inoculated control). There was an increase in proteins in all treatments, from 38.48 mg/ml to 80.58 mg/ml with the disinfectant Amruth and 66.25 mg/ml for disinfectant Ankush, while disinfectants LC, Vijatha and FL also, tend to increase the proteins content to 52.57, 48.57 and 40.27 mg/ml, respectively. In general, the highest increasing in the total proteins content recorded with Amruth and Ankush compared to inoculated control. The effect of the five disinfectants indicates the order of biochemical changes in proteins content in haemolymph of silkworm as: Amruth (80.00) > Ankush (66.25)> LC (52.57)>Vijatha (48.57) > FL (40.27 mg/ml) compared to inoculated control (38.48 mg/ml). According to the obtained results, In general, pathogen *B. bassiana*significant decreased in proteins content, but treatment of the infected mulberry silkworm;*B. mori*by disinfectants increased the proteins content as compared to the infected control. The results are in agreement with El-Sayed *et al*.(1990) who stated that, disinfectants increased the amount of haemolymph proteinsof larval instars of silkworm *B. mori*. Mallikarjuna *et al*. (2002) showed that the systematic fungicide tend to increased the total proteins content ofinfected silkworm larvae with *B. bassiana* compared to inoculated control.

Total Carbohydrates content:

The represented results in Table (1) exhibited that, infection of *B. mori* with *B. bassiana* reduced the carbohydrates content in haemolymph from 55.77 mg/ml (normal control) to 26.94 mg/ml **(**inoculatedcontrol). Elevation of total carbohydrates content after treatment by all disinfectants was noticed in haemolymph of inoculated larave. The highest elevation was recorded with disinfectant Vijatha (from 26.94 mg/ml to 85.61mg/ml), while the lowest elevation was recorded with disinfectant Amruth (26.94 mg/ml to 39.63mg/ml). There was gradual increase in carbohydrates content with all five disinfectants compared to inoculated control.The effect of the five

disinfectant indicates the order of total carbohydrates elevation in haemolymph of the infected silkworm as: Vijatha (85.61) > FL (66.51) > Ankush (48.52) > LC (42.74) > Amruth (39.63 mg/ml) compared to inoculatedcontrol (26.94 mg/ml).

Mallikarjuna *et al*. (2002) stated that, the total carbohydrates content of infected silkworm *B. mori* was decreased steadily as the disease developed and they noticed that after treatment with fungicide, the carbohydrates content increased compared to inoculated control.

Total lipids content:

The effect of disinfectants on haemolymph total lipids content in silkworminfected with *B. bassiana* is presented in Table (1).Total lipids content of inoculated haemolymph was significantlyincreased with the all disinfectant treatments except with disinfectant FL. The haemolymph total lipids content of normal control decreased from 45.57 to 35.47mg/ml (inoculated control). The highest increase was achieved withdisinfectant Ankush (from 35.47 of inoculated control to 60.00 mg/ml). Mallikarjuna *et al*. (2002) found that, there was gradual decrease in lipids content compared to normal control, while there was gradual increase in lipids content compared to inoculated control.

The biochemical parameters such as, proteins, carbohydrates, lipids, nucleic acids *etc*., are essential constituent of living cells and play a crucial role in all biological processes and vary significantly during the life cycle of all living organisms. Rajitha *et al*. (2013) reported that, inoculation of fungal pathogen resulted ingradual reduction of proteins, lipids and carbohydrates contents in the haemolymph during the progress of *B. bassiana*. The elevation of proteins, lipids and carbohydrates contents in the haemolymph of infected silkworm *B*. *mori*after treatment with the different disinfectants is very important for the chemical energy (for synthesis of ATP) which essential for life activities of insects and this elevation support the defense of *B*. *mori*against fungi and decreasing the disease development. Siraj *et al.* (2007) suggested that as energy is a vital force in the biological system, breakdown of organic constituents mainly carbohydrates is required to meet the energy under the stress condition. The increase in total carbohydrates content is indicative of preponderance of carbohydrates metabolism for energy supply to both host and pathogen. Rajitha *etal*. (2013) and Mallikarjuna *et al*. (2002) assume that the carbohydrates and lipids were used as a source of energy required for the growth and development of fungus. According to these two conclusions, increasing of proteins, lipids and carbohydrates contents in silkworm *B*. *mori*tend todecrease these contents in fungi *B. bassiana* andconsequently decreasing their efficiency infection.

Effect of disinfectants on carbohydrates hydrolyzing enzymes.

Present results in Table (2)showthat, the pathogen *B. bassiana*significant decreased in the three hydrolyzing enzymes compared to normal control. Only disinfectant LC significant increased trehalase enzyme activity from133.80 (inoculated control) to 513.15mg/ml while, the other disinfectants Ankush, Amruth, FL and Vijatha significant decreased trehalase enzyme. Also, there was an increase in amylase and invertase with disinfectant FL compared to inoculated control, while, the other disinfectants Ankush, Amruth, FL and Vijatha approximately significant decreased the two enzymes. In general, disinfectant significant decreased the activity of carbohydrates hydrolyzing enzymes.This support ourthe finding that carbohydrates were increased with application of disinfectant on infected silkworm.The decrease in activity of carbohydrates hydrolyzing enzymes tends to an increase in the carbohydrates contents.

Enzymes serve a wide variety of functions, act as catalysts and help in complex reactions occur everywhere in life. These provide the energy needed for metabolic reactions essential to immune health. Increase or decrease in enzyme activity can be correlated with the biochemical components of haemolymph of silkworm. These results may suggest that the disinfectants may affect on the enzyme activity which influences the biochemical contents of the haemolymph of the silkworm, *B*. *mori*. Inconformity with the above, in the present study, the activity of carbohydrates hydrolyzing enzymes was decreased and consequently, the total carbohydrates content increased, this elevation support the defense of *B*. *mori* against fungi and reducingthe disease development.

CONCLUSION

The present results indicated that fungi *B. bassiana* caused significant decreasing in the total proteins, lipids and carbohydrates contents in the haemolymph of infected silkworm *B*. *mori*. Application of the five disinfectants to the infected silkworm significantly increased total proteins, carbohydrates and lipids contents compared to the infected control. Elevation of these contents increased the defense of silkworm against the disease development offungi *B. bassiana*.

Table 1. Effect of five disinfectants on carbohydrates, lipids and proteins contents (mg/ml) in haemolymph ofsilkworm *B. mori* infected with fungal pathogen *B. bassiana*.

Treatments	Carbohydrates	Lipids	Proteins
Control (normal)	55.77±4.73^{c}	45.57±3.61^{c}	58.48±3.60^{b}
Control (inoculated)	26.94±3.60^{e}	35.47±3.51^{cd}	38.48±1.52^{d}
LC	42.74±3.00^{d}	44.13±3.60^{c}	52.57±3.51^{bc}
Ankush	48.52±4.35^{cd}	60.00±3.00^{a}	66.25±1.52 ^{ab}
Amruth	39.63±1.53^{d}	46.07±3.51^{c}	80.58±3.00^{a}
FL	66.51±3.00^{b}	25.00±1.53^{d}	40.27±2.08 ^{cd}
Vijatha	85.61±5.51^{a}	55.07±2,00^{b}	48.57±3.21^{c}
LSD $_{0.05}$	**5.21**	**6.19**	**7.22**

Means with the same letter(s) are non significantly different. (P<0.05). (Means±SD).

Table 2. Biochemical changes in trehalase, amylase and invertase activities (mg/ml) in haemolymph of silkworm *B. mori* inoculated with fungal pathogen *B.bassiana* and five disinfectants.

Treatments	Trehalase	Amylase	Invertase
Control (normal)	307.55±7.94^{b}	339.25±5.77^{b}	267.50±5.29^{b}
Control (inoculated)	133.80±7.77^{c}	277.74±5.77^{c}	240.50±3.05^{c}
LC	513.15±5.69^{a}	109.45±5.29^{f}	244.10±5.77^{c}
Ankush	125.20±2.52^{c}	159.45±5.77^{e}	185.00±7.21^{d}
Amruth	101.05±5.03^{d}	193.55±3.46^{d}	244.00±5.00^{c}
FL	75.05±8.50^{f}	428.90±4.36^{a}	338.30±3.21^{a}
Vijatha	131.15±5.03^{c}	275.85±8.083^{c}	232.41±4.04^{c}
LSD $_{0.05}$	**7.22**	**7.97**	**8.73**

Means with the same letter(s) are non significantly different. (P<0.05). (Means±SD).

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تأثير خمس من المطهرات(مضادات الفطريات)علي بعض التغيرات الكيميائية الحيوية، في هيموليمف دودة الحريرالتوتية المصابة بالفطر *Beauveriabassiana*

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في هذا البحث تم دراسة تأثير خمسة أنواع من المطهرات (مضادات الفطريات) كيميائية ونباتية: {LC(نباتي) Amruth (نباتي) FL (كيميائي) Vijatha (كيميائي) Ankush (نباتي كيميائي)} علي بعض التغيرات الكيميائية الحيوية في هيموليمفدودة القز التوتية المصابة بالفطر الممرض *Beauveria bassiana*.

وقد أوضحت النتائج أن الفطر الممرض لدودة القز التوتية أدي إلى إنخفاض محتوى البروتينات والكربوهيدرات والدهون وكذلك نشاط الانزيمات المحللة للكربوهيدرات (Trehalase, amylase and invertase) مقارنة بالكنترول الطبيعي، وقد أظهرت الدراسة أيضا أن استخدام المطهرات أدي إلى ارتفاع محتوى البروتينات و الكربوهيدرات والدهون مقارنة بالكنترول المعدي بينما انخفض نشاط الانزيمات المحللة للكربوهيدرات ما عدا المطهر LCأدي إلى زيادة نشاط انزيم Trehalase و المطهر FL أدي إلى زيادة نشاط انزيمي amylase and invertase.

طبقا لهذه الدراسة يمكن أن إستنتاج أن استخدام هذه المطهرات يؤديإلي ارتفاع محتوى البروتينات و الكربوهيدرات والدهونوهذا الإرتفاع يقوي من دفاع دودة القز التوتية ضد الفطر الممرض ويقلل من تطور المرض. لذلك نوصي بإستخدام هذه المطهرات في مكافحة الأمراض في صناعة الحرير في مصر لأنها رخيصة، طبيعية و صديقة للبيئة بعد إجراء التجارب البيولوجية المطلوبة.