



## INFLUENCE OF SOME PLANT EXTRACTS AS ANTISEPTICS TO CONTROL BACTERIAL AND FUNGAL DISEASES OF SILKWORMS, *Bombyx mori* L.

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**ABSTRACT:** This study was carried out to investigate the influence of petroleum-ether extracts of mulberry leaves (*Morus alba*), basil leaves (*Ocimum basilicum*), and black seed (*Nigella sativa*) as disinfectants on some biological and physiological aspects of the mulberry silkworm, *Bombyx mori* L. larvae artificially infected with *Bacillus thuringiensis* and *Beauveria bassiana*. The results showed that silkworm larvae artificially infected with *B. thuringiensis* using black seed and mulberry leaves extracts (2, 3%) produced the highest cocooning percentage (98%) while using (3%) basil leaves extract enhanced female fecundity (634 egg/female). Also, the highest total soluble protein in hemolymph recorded 8.5 mg/ml when using 1% of Black seed and Basil leaves extracts with infected larvae with *B. thuringiensis*. Otherwise, silkworm larvae artificially infected with *B. bassiana* using black seed extract 3% led to the highest cocooning percentage (98%) and maximum egg number deposited (613 egg/female). In addition, all plant extracts under study increased the activity of transaminase enzymes. Moreover, concentrations of basil leaves extract increased the total protein in haemolymph of infected larvae with *B. bassiana*.

**Key words:** *Bombyx mori*, *Bacillus thuringiensis*, *Beauveria bassiana*, mulberry leaves, basil leaves, black seed.

## INTRODUCTION

The mulberry silkworm, *B. mori* L. is susceptible to infection of several microbial pathogens resulting in a heavy loss in the silk productivity. Diseases of the silkworm, *B. mori* seriously affect their cocoon production, among which bacterial pathogens independently cause cocoon loss to the tune of 75 percent. Investigation of the hemolymph collected from the diseased silkworm revealed that the cultured colony was that of *Staphylococcus* sp. according to its morphological characters (Karthikairaj *et al.*, 2014). Microbes such as *Bacillus* sp, *Staphylococcus acire*, *Staphylococcus epidermidis* and *Streptococcus faccalis*, *Streptococcus liquifactions* are commonly detailed to cause

bacterial infections in silkworm (Karthikairaj *et al.*, 2013). Besides, a dozen species of fungi cause infections in silkworm, such as the members of the genera *Beauveria* and *Metarrhizium* are of great importance, which causes muscardine disease. However, bacterial infection is well controlled by antibiotics: The ability of bacteria to develop resistance to bactericides make it ineffective within a short time and hence attempts made for the application of plant extracts against silkworm bacterial pathogens (Priyadharshini *et al.*, 2008).

The use of botanicals having anti-microbial property, non-toxic, biodegradable, and non-pollutant, is an alternative strategy to control diseases of silkworm. The losses occur mainly during the final stages of silkworm rearing,

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resulting considerable energy and money loss. The effects 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, as well as on the cocoon economic aspects (**Rajitha and Savithri, 2015**). Many higher plants produce organic compounds, which possess antimicrobial activities. These compounds have advantages over synthetic bactericides and fungicides like environmentally friendly, target specificity, to overcome disease resistance and easy availability. Phytochemicals are compounds present in plants and have antioxidants, antiviral, antibacterial, antifungal, anticancer activities, or hormone-like components that help in fighting against many diseases and maintain health (**Akinmoladun *et al.*, 2007; Daniel *et al.*, 2011**).

*Nigella sativa* L., black seeds has many different chemical ingredients that possess many medical properties including immune-modulatory activities, as well as, anti-inflammatory, antimicrobial and antioxidative effects (**Ahmad *et al.*, 2013; Ishtiaq *et al.*, 2013; Tembhurne *et al.*, 2014**).

*Morus alba* L. has been identified as a versatile multifunctional plant with enormous economic, nutritional and health potentials. Leaves extract of *M. alba* is reported to contain the main active principles Phyto-constituents of alkaloids, phlorotannins, phenols, quinones, cardiac glycosides, carotenoids, reducing sugars and volatile oils (**El-Beshbishy *et al.*, 2006; Sabira and Shail, 2016**). As well, some phenolic compounds, flavonoids, stilbenes, and 2-arylbenzofurans have been known to show antimicrobial effect (**Shivanna and Raveesha, 2009**).

Furthermore, *Ocimum basilicum* L. is an important medicinal plant that contains several antimicrobial compounds (**Politeo *et al.*, 2007; Zhang *et al.*, 2009**). The essential oil of *O. basilicum* contains methyl eugenol, methyl chavicol, monoterpenes and phenylpropanoids which have bactericidal and antifungal properties.

In the present study, we investigated the effect of petroleum-ether extracts of mulberry leaves, basil leaves and black seed as disinfectants on some biological and physiological aspects of the silkworm, *B. mori* larvae artificially infected with *B. thuringiensis* and *B. bassiana*.

## MATERIALS AND METHODS

### Materials and Chemicals

Mulberry leaves (Balady variety) were collected from Zagazig District, Egypt. Formaldehyde (37%), petroleum-ether (40/60 V/V), and phenylthiourea were purchased from Sigma Aldrich Chemical Corporation, Egypt. (ALT) Alanine aminotransferase, Aspartate transaminase (AST) and Bradford protein assay kits were purchased from El-Gomhouria Co. For Trading Drugs, Chemicals and Medical Supplies, Egypt.

### Insect Source and Rearing

Silkworm hybrid eggs of (G2×V2×KK×H1) were obtained from the Sericulture Research Department (SRD), Plant Protection Research Institute (PPRI), Agricultural Research Center, Egypt, and maintained in rearing room at (28 ± 2°C, 70 ± 5% RH. and 14:10 hr., light : dark) according to the technique of **Krishnaswami (1978)**. Mulberry leaves were collected twice daily, *i.e.* at 8 am and 4 pm, then washed and left to dry as needed under room conditions. Larvae were offered mulberry leaves 4 times/day on plastic trays (42 x 30 x10 cm) with the number of 100 larvae/tray. Rearing trays, tools, and rearing rooms were disinfected one-week prior the onset of the experiment using 3% formalin. The larval bed was changed daily using cleaning net for removing the remained dried food and feces. Chicken egg carton plates were used as montages for cocoon spinning (**Zannoon and Omera, 1994**).

### Isolation of Bacteria

Bacterial isolation and identification was carried out in Physiology Department, ARC, Egypt. Bacterial pathogens were collected from black thorax Septicemia diseased larvae. The diseased larvae were crushed by using mortar and pestle. The homogenate was then filtered with silica filter. The filtrate was centrifuged at 5000 g for 10 min. The supernatant was discarded, and the pellet was used for bacterial culture after re-suspending in distilled water (**Aneja, 2007**).

## Preparation and Count of Bacterial Culture

*B. thuringiensis* was cultured overnight in nutrient agar medium at 28°C. Then, the bacterial colonies were selected and transferred into 10 ml NaCl (0.85%) as stock solution. Cells were counted in 100 ul using flow cytometry. The cell no. 50 cells the total cells in 1 ml equal 500,000 cells. Serial dilution with different concentration of bacterial spore suspensions were made by take 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µm from stock solution and complete each one to 1 ml by saline for making the different concentrations 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% (Cockerill *et al.*, 2012).

## Fungal Isolation and Preparation

The fungus was isolated from silkworm larvae died from white muscardine using Sabouraud Dextrose Agar medium according to Meyling and Eilenberg (2006) and Meyling (2007). Culture of *B. bassiana* was maintained on Potato Dextrose Agar slants and was subcultured in Petri dishes according to Linday (1962). The conidial concentration ( $1 \times 10^7$  conidia/ml) was estimated using Neubauer hemocytometer under a Leitz Dialux 20EB microscope (400x magnifications) according to Yeo *et al.* (2003).

## Infecting of Silkworm Larvae with Bacteria and Fungi

After preparing bacterial spore suspension, *B. mori* larvae artificially infected by spraying the mulberry leaves with concentration (50%) of bacterial suspension (*B. thuringiensis*) one time in the 2<sup>nd</sup> day of the 4<sup>th</sup> instar larvae (Hassan, 2015). As for the fungus infection, the larvae were immersed in the prepared fungus solution for 10 sec.

## Experimental Design and Larval Treatment

The plant extracts were prepared from crushed of mulberry leaves (*M. alba*), basil leaves (*O. basilicum*), and black seeds (*N. sativa*). Then, the dry powdered plants were macerated in petroleum-ether (40/60 V/V) according to Harborne (1984). Moreover, after the third ecdysis; the larvae were divided into three groups. The first group served as control. The second group was fed once in the 2<sup>nd</sup> day of

the 4<sup>th</sup> instar larvae on mulberry leaves treated with concentration (50%) bacterial spore suspension of *B. thuringiensis*. Finally, the third group larvae were dipped for 10 sec once with conidial solution of *B. bassiana* fungus. Thus, the infected groups (2 and 3), each one divided into 4 sub-groups, which treated with the three concentrations 1, 2, and 3% of *M. alba*, *O. basilicum* leaves extracts, and *N. sativa* seeds extract, and the other one remained without extracts treatment (infected control). All these concentrations were applied as bed disinfectant sprays on mulberry leaves and larvae three times during each of fourth and fifth larval instars. Three replicates of 100 larvae each were used for each concentration.

## Biochemical Determination

Hemolymph samples were obtained by removing one of the thoracic legs of the 5<sup>th</sup> instar larvae and bending the body to expose the sternum at the position of the removed leg. To ensure proper drainage of the hemolymph to avoid any risk of internal organs to be destructed. The hemolymph of each treatment was collected in Eppendorf tubes 1.5 ml containing a few crystals of phenylthiourea (PTU) to prevent melanization of samples (Mahmoud, 1988). The tubes were kept at -20°C. The blood samples were centrifuged at 10000 rpm for 10 minutes at 5°C. The supernatant was immediately assayed to determine AST, ALT activities according to the method of Reitman and Frankel (1957), total soluble protein (TSP) as described by Gornall *et al.* (1949) and total carbohydrate fractions according to Ishaaya and Swirski (1976).

## Developmental Measurements

Different biological characteristics were measured such as cocooning percentage, adult emergence percentages, fecundity (total number of laid eggs/female) and fertility percentage.

## Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA); means were compared using Duncan's test according to Snedecor and Cochran (1982) using Costat V.6.311 (2005) Software.

## RESULTS

### Effects of Plant Extracts on Biological and Physiological Aspects of *B. mori* larvae artificially infected with *B. thuringiensis*

#### Biological aspects

Results presented in Table 1, reveal that the highest cocooning percentages 98.0, 96.0 and 97.0% were recorded for infected larvae treated with the mulberry leaves, black seed, and basil leaves extracts (3%), respectively. Feeding *B. mori* larvae on mulberry leaves treated with a spore suspension of *B. thuringiensis* (Infected control) recorded cocooning percentages of 83.3% compared with 92.0% for uninfected larvae. Statistical analysis revealed significant differences in cocooning percentage means. Concerning emergence percentages, obtained results cleared that the infection of silkworm larvae with bacterial spore suspension, decreased the emergence percentage of moths to 75.0% in comparison with 96.0% for normal control.

The treatment with higher concentrations (3 and 2%) of the tested extracts increased emergence percentage of moths as compared to the untreated control (Table 1).

The highest mean number of deposit eggs were 602, 595, and 634 egg/female for the moths resulted from infected larvae treated with 3% mulberry leaves, black seed, and basil leaves extracts, respectively. Meanwhile, the least mean (444 egg/female) was recorded for *Bacillus* infected control (Table 1). The differences in fecundity means among different concentrations are highly significant. Regarding egg fertility percentages, the differences between means are significant. Feeding infected silkworm larvae on mulberry leaves treated with 1.2% mulberry leaves extracts, and 3% concentration of black seed and basil leaves gave the highest mean fertility 99.1, 99.7, 98.7 and 97.7%, respectively, meanwhile, *Bacillus* infected control showed the least fertility percentage 77.7% (Table 1).

Generally, using basil leaves extract as disinfectant proved to give higher cocooning, fecundity, and emergence percentages over the other tested extracts, regardless of concentration.

Meanwhile, mulberry leaves extract was superior for fertility and adult emergence percentages, regardless of concentration.

#### Physiological Parameters

As shown in Fig. 1 the highest total soluble protein content in hemolymph was 8.5 mg/ml recorded for both larval treated with concentration 1% of black seed and basil leaves extracts; while the least total soluble protein content in hemolymph (6.2 mg/ml) was recorded for *Bacillus* infected control group.

Regarding ALT enzyme, the highest activity was (60.6, 55.8 and 53.3 mg/ml) recorded from hemolymph samples taken from infected larvae treated with (3%) basil leaves, and (1, 3%) black seed extracts, respectively. On the other hand, the lowest activity was (39.0 mg/ml) recorded for infected control larvae. Obtained data clear that, the tested plant extracts under study led to increased the AST enzyme activity for infected silkworm larvae by *Bacillus*, regardless of concentration compared with the control.

In case of total carbohydrate, control larvae manifested the highest content, recording 65.9 mg/ml, while infected control larvae possessed the least total carbohydrate content (28.6 mg/ml). Other treatments gave intermediate values, ranging between 62.1 – 40.4 mg/ml (Fig. 1).

### Effects of Plant Extracts as Disinfectants on Biological and Physiological Aspects of *B. mori* Larvae Artificially Infected with *B. bassiana*.

#### Biological aspects

Tabulated results cleared that treating silkworm larvae with black seeds, basil leaves, and mulberry leaves extracts at 3% resulted in the highest cocooning percentage, recording 98.0, 97.3, and 97.0%, respectively. *B. bassiana* infected control reduced the cocooning percentage to 83.3% compared with 92.0% in the control group (Table 2). Statistical analysis of cocooning percentage data revealed highly significant differences among tested concentrations.

Results indicated that the used plant extracts under study with concentrations 2 and 3% as

Table 1. Effect of mulberry leaves, black seed, and basil leaves extracts as disinfectants on biological aspects of artificially infected silkworm, *B. mori* larvae with *B. thuringiensis*

Extract	Conc. (%)	Cocooning (%)	Emergence (%)	Fecundity (Egg/female)	Fertility (%)
Mulberry leaves	1	94.3	97.0	496	99.1
	2	96.0	99.3	561	99.7
	3	98.0	97.3	602	96.6
Black seeds	1	95.7	92.7	563	88.7
	2	98.0	97.0	565	90.3
	3	96.0	99.3	595	98.7
Basil leaves	1	95.0	93.0	541	92.0
	2	97.3	97.0	608	96.7
	3	97.0	99.3	634	97.7
Infected control <i>B. thuringiensis</i>		83.3	75.0	444	77.7
Control		92.0	96.0	567	96.3
LSD <sub>0.05</sub>		2.08	2.01	82.64	2.87
P<0.05		0.0001**	0.0001**	0.0074**	0.0001**

(\*\*) denote significant differences at 0.001 levels of probability.

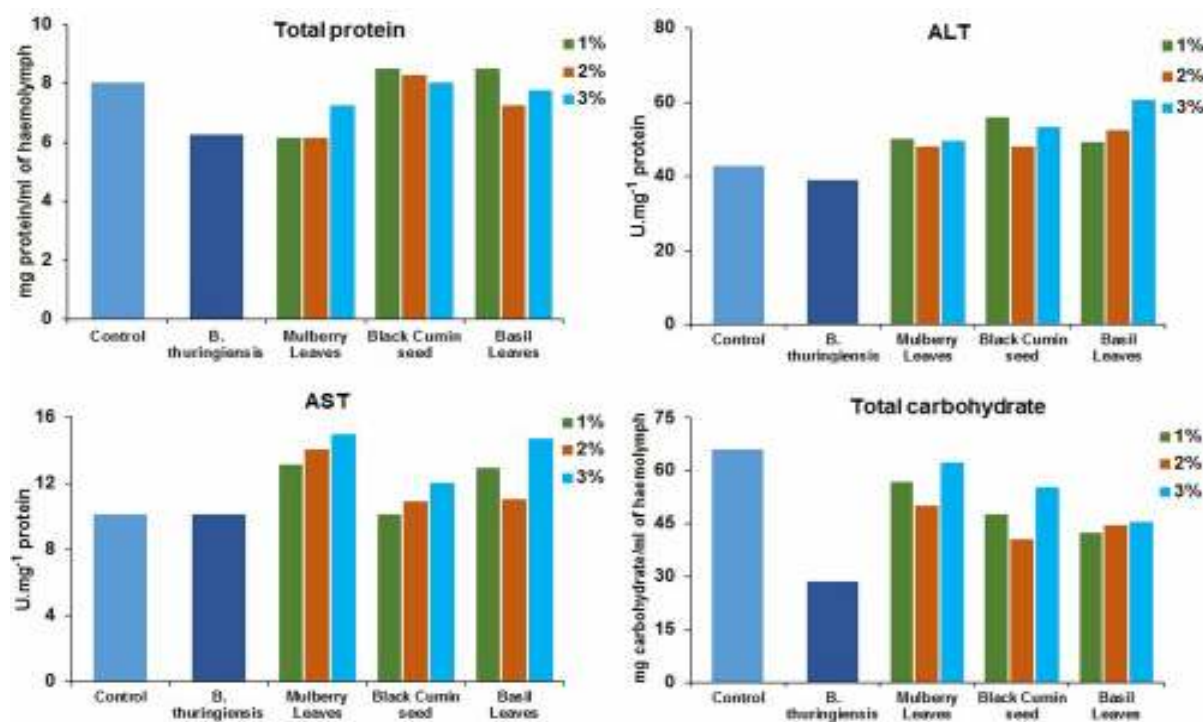


Fig. 1. Effect of mulberry leaves, black seed, and basil leaves extracts as disinfectants on physiological aspects of artificially infected silkworm, *B. mori* larvae with *B. thuringiensis*

**Table 2. Effect of mulberry leaves, black seed, and basil leaves extracts as disinfectants on some biological aspects of silkworm, *B. mori* larvae artificially infected with *B. bassiana***

Extract	Conc. (%)	Cocooning (%)	Emergence (%)	Fecundity (Eggs/female)	Fertility (%)
Mulberry leaves	1	91.0	92.0	522	90.3
	2	97.0	94.7	564	90.0
	3	97.0	97.3	603	96.3
Black seeds	1	91.7	90.0	569	93.7
	2	94.0	97.3	597	99.0
	3	97.3	99.3	613	97.3
Basil Leaves	1	91.0	89.7	560	90.3
	2	94.0	97.3	583	93.0
	3	98.0	98.3	612	99.7
Infected control <i>B. bassiana</i>		83.33	79.0	424	81.0
Control		92.0	96.0	567	96.3
LSD <sub>0.05</sub>		2.083	1.86	28.47	2.605
P≤0.05		0.0001**	0.0001**	0.0001**	0.0001**

(\*\*) Denote significant differences at 0.0001 levels of probability, respectively.

disinfectants on artificially infected mulberry silkworm with *B. Basin* increased moth emergence with means ranged between 94.7 - 99.3%, in comparison to the control group (96.0%). Statistical analysis of data revealed significant differences between means.

Regarding the number of deposited eggs/female, the maximum numbers of deposited eggs/female were 613, 612 and 603 eggs recorded for infected larvae treated with 3% of each black seed, basil leaves and mulberry leaves extracts, respectively, meanwhile, the minimum deposited eggs (424 eggs/female) was counted for moths resulted from larvae infected with *B. bassiana* conidial solution. The differences among the means were highly significant. In addition, the same trend was noticed for egg fertility percentage, where the highest fertility percentage (99.0 and 99.7%) was recorded for infested silkworm larvae treated with concentration 2% black seed and 3% basil leaves extracts, respectively. There are highly significant differences between the means of egg fertility (Table 2).

### Physiological parameters

Obtained results revealed that the application of all the tested concentrations of basil leaves extract increased total protein content in the hemolymph of artificially infected silkworm larvae with *B. bassiana*. The total protein content in haemolymph of the infected silkworm, *B. mori* larvae with *B. bassiana* recorded 7.6 mg/ml as compared to 8.0 mg/ml for normal control. Concerning ALT and AST enzyme activity, it was found that all tested plant extracts increased the activity of transaminase enzymes, showing averages of 61.6-53.0 and 13.9-11.9 mg/ml, respectively, in comparison with 42.8 and 10.1 mg/ml for normal control (Fig. 2). Meanwhile, *B. bassiana* infected control showed the least activity (35.3 and 9.9 mg/ml) for the investigated enzymes, respectively.

As shown in Fig. 2, control larvae manifested the highest total carbohydrate content recording 65.9 mg/ml; meanwhile, the least content (33.4 mg/ml) was recorded for 2% basil leaves extract, regardless of the concentration. In conclusion, plant extracts affected positively on physiological aspects as a disinfectant for mulberry silkworm *B. mori*.

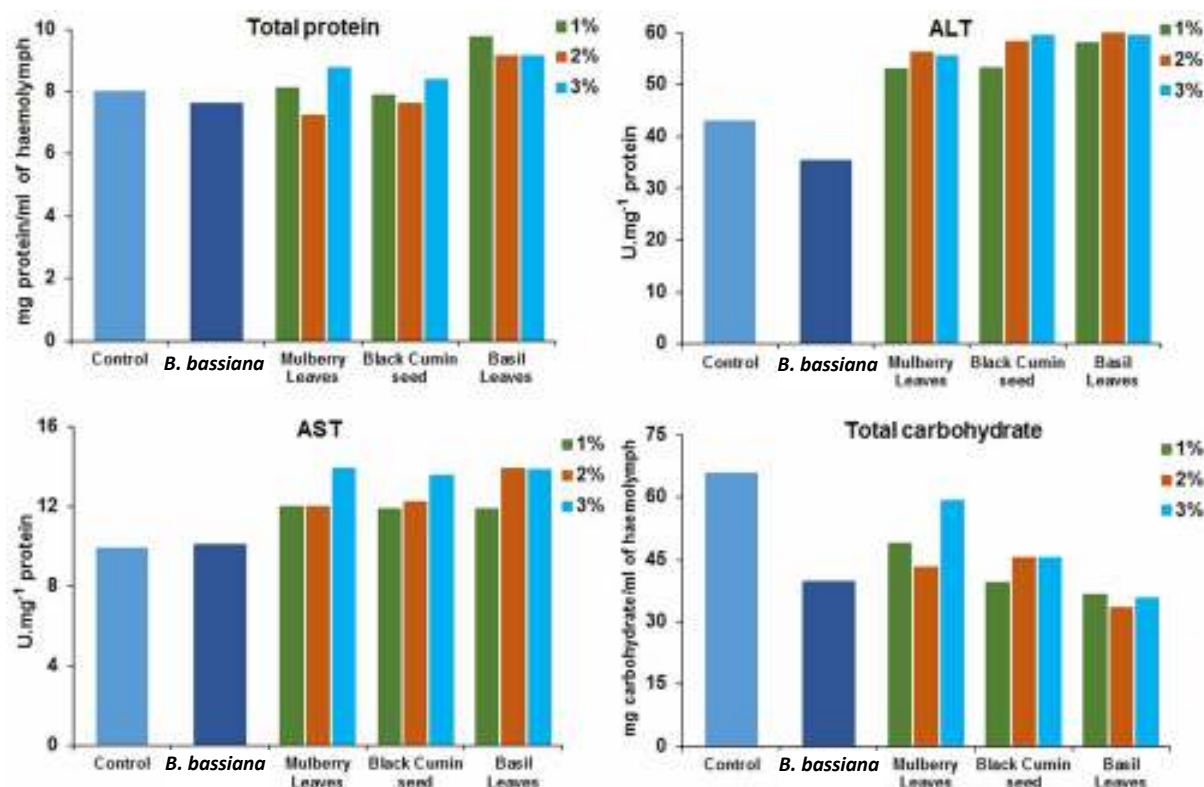


Fig. 2. Effect of mulberry leaves, black seed, and basil leaves extracts as disinfectants on some physiological aspects of silkworm, *B. mori* larvae artificially infected with *B. bassiana*

## DISCUSSION

The curing of silkworm diseases is a vital point of successful silkworm rearing for gaining a higher cocoon crop and good silk yield. Medicinal plants had a rich source of antimicrobial agents. **Beegum and Devi (2003)** stated that peppermint oil inhibited the growth of many kinds of bacteria and fungi. **Kumar et al. (2013)** reported that the extract of *O. sanctum* contains eugenol that responsible for antimicrobial activity. In addition, cinnamon oil extract showed antibacterial activity against bacteria that infect silkworm (**Omar and Fathy, 2016**).

Application of *M. alba*, *O. basilicum* leaves, and *N. sativa* seed extracts as disinfectants over infected mulberry silkworm, *B. mori* larvae with bacterial spores suspension (*B. thuringiensis*) increased cocooning percentage, the fecundity of female moth, fertility percentage and emergence of adult moths. The enhancement in *B. mori* characters may be due to the presence of

antimicrobial and antioxidant constituents in mulberry leaves, basil leaves, and black seeds extracts.

This finding is in agreement with that of **Venkatesh and Srivastava (2010)**, who reported that mulberry leaves supplemented with 0.1% cloxacillin, terramycin (oxy-tetracycline), streptomycin or 0.5% gentamycin were effective in improving the growth of *B. mori* race Nistari. The same results were obtained by **Hassan and Saad (2012)**, who proved that fecundity increased when the silkworm, *B. mori* larvae treated with the volatile oils. Also, they stated that the fecundity of female moths significantly increased with germ wheat oil treatment. Moreover, obtained results were similar to those obtained by **Gad (2006 and 2013)** who found that treatment with honeybee products increased the number of deposited eggs/female than those obtained from control. Also, Cefotax (400 ppm) gave the highest cocooning percentage when used at fourth and fifth larval instars (**Saad et al., 2012**). Application of flaxseed micro

particles emulsion resulted in maximum larval weight, pupal weight, and high number of laid eggs per female moth and fertilized eggs per female moth in 0.25% treated group significantly, compared with the other treated groups (Taha and Sadek, 2018).

Recent studies have shown that *M. alba* leaves have antioxidant, antibacterial, antiviral, and anti-inflammatory properties (El-Beshbishy et al., 2006). Some phenolic compounds (flavonoids, stilbenes, and 2-arylbenzofurans) have been reported from *M. Alba* and have been known to show antimicrobial effect (Shivanna and Raveesha, 2009).

The antibacterial activity of black seeds extract is due to the presence of thymol, which is responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through nonspecific interactions with the proteins (Gsmalseed et al., 2016). Application of *Ankush vijetha* green and *Slaked lime* powder combination as per recommended schedule leads to low muscardine incidence during winter season and increased single cocoon weight, shell weight, pupal weight, shell percentage, filament length and cocoon yield (Shashidhar and Thulasiram, 2018). Also, black seeds oil extracted by methanolic, ethanolic, diethyl ether, acetonetic and n-hexane shows effective antibacterial activity against both gram-positive and gram-negative bacteria (Ishtiaq et al., 2013). Moreover, Karthikairaj et al. (2013) proved the antibacterial activity of aqueous and alcoholic extracts of *O. Sanctum*, *A. Paniculata*, and *M. charentia* for the control of flacherie disease and thereby improved the silk production. Karthikairaj et al. (2014) proved that both aqueous and alcoholic extracts of *Acalypha indica*, *Leucas aspera*, and *O. sanctum* were effective against *Staphylococcus* sp. infecting mulberry silkworm *B. mori*.

Finally, the use of bed disinfectants is very important to control the diseases in silkworm rearing and to gain high cocoon crop and silk production. El-Yamani et al. (2017) indicated that *Cupressus macrocarpa* oil, when offered to *B. mori* larvae at all tested concentrations, increased cocooning percentage as compared to control. So the application of *Ankush vijetha*

green and *slaked lime* powder combination (T3) as bed disinfectant resulted in lower incidence of silkworm diseases like flacherie (0.25 - 0.15%) and muscardine (0.05 - 0.35%) (Shashidhar and Thulasiram, 2018).

The results obtained by Azab (2003) proved that the infection with *B. thuringiensis* caused a marked reduction in the total protein contents in different concentrations in the black cutworm.

In the case of mulberry silkworm infection with a colloidal solution of *B. bassiana* it was found that the treatment of the investigated plant extracts increased cocooning, fertility, emergence percentages, and increased the number of deposit eggs/female. Swathi et al. (2014) obtained similar results, who stated that the incidence of muscardine disease occurred by daily application of active lime powder at the rate of 3g/sq ft and hydrated lime powder at the rate of 5g/sq ft in combination with bundh powder after every molt. These results are in concurrence with Taha et al. (2016), who obtained the highest larval weight and cocoon weight after application of LC (mix of *Lawson iainermis* and *Curcuma longa* powders). Cocooning percentage was significantly high in FL (Formaldehyde and lime powder 0.25:0.75) and LC treatments as compared to inoculated control group (immersed in fungal spores solution).

According to the obtained results, using the plant extracts to the infected mulberry silkworm, *B. mori* increased the protein content, ALT and AST activities, and total carbohydrate content as compared to the infected control group. The infection with bacterial diseases decreased the total protein content. Also, Hassan (2015) proved that using the plant oils and antibiotics to the infected mulberry silkworm, *B. mori* increased the protein content and stimulated ALT and AST enzyme activity as compared to the infected control group. El-Yamani et al. (2017) said that total soluble protein content significantly increased by the addition of 1% *L. sativum* and *C. Macrocarpa* oils. The antifungal activity may be contributing to the presence of biologically active compounds to like cinnamyl alcohol, caryophyllene, thymoquinone, palmitic acid, and linoleic acid (Vadivel and Gopalakrishnan, 2011; Sermakkani and Thangapandian, 2012).



## Conclusion

Hygiene for silkworm breeding is important to save money and silk production by avoiding infection and fighting bacterial and fungal diseases. Using the plant extract of mulberry leaves, black seed and basil leaves led to an improvement in some important biological and physiological parameters under study, such as the cocooning and pupation percentage and emergency percentage as well as the number of eggs laid by the female. In addition, the increased activity of total protein and enzyme activity (ALT and AST); while, reduced the presence of total carbohydrates, which led to an improvement in the resistance of bacterial and fungal diseases. Therefore, we recommend the use of mulberry, basil and black bean extracts as natural and safe disinfectants for rearing the mulberry silkworm.

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## تأثير بعض المستخلصات النباتية المستخدمة كمطهرات للسيطرة على الأمراض البكتيرية والفطرية التي تصيب دودة الحرير التوتية *Bombyx mori* L.

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أجريت هذه الدراسة في معمل قسم بحوث الحرير بمعهد بحوث وقاية النباتات بمركز البحوث الزراعية خلال موسم الربيع ٢٠١٨ لاستعراض تأثير مستخلصات الأثير البترولي لأوراق التوت، الريحان وبذور حبة البركة كمطهرات علي بعض الصفات الفسيولوجية لدودة الحرير التوتية *Bombyx mori* التي تم عدوها صناعياً بالبكتريا *Bacillus thuriangiensis* وفطر *Beauveria bassiana*، سجلت أعلى نسبة تشرنق (٩٨,٠٠%) في حالة يرقات *B. mori* المصابة بالبكتيريا *B. thuriangiensis* والتي عولجت بمستخلصات نبات التوت تركيز ٣% أو مستخلص بذور حبة البركة تركيز ٢%، لوحظ أعلى عدد للبيض الموضوع (٦٣٤ بيضة/فراشة) للفراشات الناتجة عن يرقات مصابة عولمت بتركيز ٣% بأوراق نبات الريحان، إثراء أوراق التوت بالمستخلصات النباتية قيد الدراسة بالتركيزين ٢ و٣% أحدثت زيادة في نسبة خروج الفراشات مقارنة بالكنترول المصاب أو غير المعالج، هذا وقد بلغت أعلى نسبة محتوى اليرقات من البروتينات الذائبة في الهيموليمف (٨,٥٠٠ ملجم/مل) لكل من اليرقات التي تغذت على أوراق توت معاملة بمستخلصات بذور حبة البركة بتركيز ١% وأوراق الريحان بتركيز ١%، أما في حالة الكريوهيدرات الكلية فقد وجد أن يرقات المقارنة قد أظهرت أعلى محتوى لها، بالنسبة ليرقات *B. mori* المصابة صناعياً بفطر *B. bassiana*، فقد وجد أن معاملة أوراق التوت بمستخلص بذور حبة البركة ٣% قد نتج عنها أعلى نسبة تشرنق حيث بلغت ٩٨,٠٠% وأعلى عدد للبيض الموضوع (٦١٣ بيضة/فراشة)، جميع المستخلصات النباتية قيد الدراسة زادت من نسبة خروج الفراشات ونشاط الإنزيمات الناقلة للأمين، جميع التركيزات المختبرة لأوراق الريحان أدت إلى زيادة إجمالي محتوى البروتينات الذائبة في الهيموليمف ليرقات دودة القز المصابة صناعياً باستخدام مسببات العدوى *B. bassiana*.

المحكمون:

رئيس بحوث وقاية النباتات بمركز البحوث الزراعية فرع الشرقية.  
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