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Controlling of bacterial and fungal diseases that contaminating mulberry silkworm, *Bombyx mori* by using some plant extracts

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Abstract

Background: Mulberry silkworm, *Bombyx mori* L. is one of the most economically important insect because its production of silk which interfere with many important industries. Diseases of the silkworm, *B. mori* such as viral, bacterial, fungal and protozoan pathogens seriously affect their cocoon production. Bacterial and fungal pathogens independently cause the highest cocoon loss, so the use of botanicals is considered an important strategy to control diseases of silkworm.

Results: The artificially infected silkworm, *B. mori* larvae with *Bacillus thuriangiensis* when treated with concentrations of 2 and 3% of black seed and basil leaves extracts increased significantly larval weight and decreased larval mortality. In addition, 3% basil leaves extract and 1% of black seed increased pupal weight. Cocoon weight and cocoon shell weight increased significantly by 3% basil leaves extract treatment. All tested concentrations of black seed extract increased the silk ratios of the resulted cocoons as compared to the infected control. For technological parameters, all tested extracts at 3% concentration caused the highest silk filament length; meanwhile control larvae recorded the highest silk filament weight and size. Regarding the mulberry silkworm, *B. mori* artificially infested with conidial solution of *Beauveria bassiana* when treated with all concentrations of the tested plants showed an enhancement in larval weight, pupal weight, cocoon weight, cocoon shell weight, silk filament length, and filament weight and reduced the larval mortality percentage. The highest silk filament size were recorded for the control group.

Conclusions: Tested concentrations of *Morus alba*, *Ocimum basilicum* leaves extracts and *Nigella sativa* seeds extract have suppressed the bacterial and fungal disease spread when used three times through both 4th and 5th larval instars. Moreover, the biological characters and technological traits enhanced by using of these extracts, so it can be used in sericulture for improving cocoon crop quality and quantity.

Keywords: *Bombyx mori*, *Bacillus thuriangiensis*, *Beauveria bassiana*, Artificially infection, Biology, Technology, Silk filament

Background

Natural silk is produced by the silkworm, which is used for many purposes. Silk thread has several uses, including parachute manufacturing, tire lining, electrical insulation, artificial blood vessels, surgical thread. Oil can be extracted from silkworm pupa, amino acids and vitamin B2 and can be used in the pharmaceutical industry. Phytol, an important raw material to make vitamin E and K, and colorful can be extracted from the faces of

the silkworm. The faces can also used to make activated carbon and acid-resistant plastic sheets, rich in protein, the silkworm faces are good feeds for fish, pigs, cattle and sheep. It is super organic manure (Souad Mahmoud (2016).

Overall, the silkworm industry plays an important and active role in the development of national income and stimulates the rural economy and the overall development of agriculture, forestry, animal husbandry, side-line occupations and fisheries, suprs the development of local light industry and expands trade (Dandin 2014).

The mulberry silkworm, *B. mori* produces silk, which are influenced by many pathogens includes fungi,

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bacteria, virus and protozoan which cause damages to cocoons reached to 75% (Das 1950). The frequency of infections at silkworm breeding extremely diminishes the producing of silk. Microbes such as *Streptococcus faecalis*, *Streptococcus liquifactions*, *Staphylococcus acire*, *Staphylococcus epidermidis* and *Bacillus* sp. are commonly detailed to cause the bacterial flacherie in silkworm (Karthikairaj et al. 2013). The misfortunes happen basically amid the ultimate stages of silkworm raising coming about impressive vitality and cash loss.

The biodiversity of plants gives a potential source of the antimicrobial agents (Bushra and Ganga 2003), which may be applied for antiviral, antibacterial and antifungal effectiveness (Pereira et al. 2004; Kartal 2007). *Morus alba* L., known as white mulberry, has a short-lived, fast-growing, little to the medium tree. The last studies appeared *M. alba* has antioxidant, antibacterial, antiviral and anti-inflammatory properties (El-Beshbishy et al. 2006). Some phenolic compounds flavonoids, stilbenes, and 2-arylbenzofurans have been detailed from *M. alba* and have been known as antimicrobial (Yogisha and Raveesha 2009). *M. alba* has been distinguished as a multifunctional flexible plant with colossal dietary and wellbeing possibilities. The antimicrobial movement of the ethanolic and water extricates of *M. alba* variations appeared a few inhibitory control against the organisms like *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae* and *Proteus vulgaricus*, *Staphylococcus aureus* and *Streptococcus faecium* for gram negative and gram positive microscopic organisms and against *Aspergillus tamari*, *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium oxalicum* for organisms (Omidiran et al. 2012).

In spite of the fact that dark seed extract or oil has been detailed to have antimicrobial validity (Morsi 2000). *Nigella sativa* have pharmacological activities was investigated as anticancer, antimicrobial, anti-inflammatory, hepatoprotective, renal defensive, gastroprotective, antioxidant properties Desai et al. (2015). Numerous dynamic compounds have been separated and distinguished from dark seeds (Ahmed et al. 2013). These compounds were thymoquinone (30%–48%), thymohydroquinone, dithymoquinone, p-cymene (7%–15%), carvacrol (6%–12%), 4-terpineol (2%–7%), t-anethol (1%–4%), sesquiterpenelongifolene (1%–8%) α -pineneand thymol etc.

Sweet basil (*Ocimum basilicum* L.) belongs to the family Lamiaceae, its constituents have antibacterial, antifungal, antioxidant and radio protective activity (Kath and Gupta 2006; Ramesh and Satkohan 2010). Prasad et al. (2012) indicate that, *Ocimum* possesses compounds with antimicrobial properties against pathogenic microorganisms. *O. sanctum* extract has growth promoting effect on silkworm, which helps to enhance the commercial qualities of silk and can be used in sericulture for yield improvement.

Aim of work

The present study was carried out to evaluate the effect of some plant extracts to controlling bacterial and fungal diseases of mulberry silkworm *B. mori* larvae and their impact on some biological and technological aspects.

Material and methods

Rearing of mulberry silkworm

Silkworm eggs of commercial Bulgarian hybrid were used (H1*KK*G2*V2) and obtained from Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Center (ARC) and maintained in rearing room of silkworm under laboratory conditions ($28 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ RH) according to the method described by Krishnaswami (1978). Mulberry leaves (Balady variety) were collected twice daily i.e. at 8 am and 4 pm, then washed and left to dry as needed in the room temperature. Rearing trays, tools, and rearing room were disinfected one-week prior the onset of the experiment using formalin 3% (37–40% formaldehyde). Larvae were offered to mulberry leaves 4 times/day in plastic trays ($42 \times 30 \times 10$ cm) with approximate number of 100 larvae/tray for three replicates for each concentration. The larval bed was cleaned daily using cleaning net for removing the remained dried food and feces. Chicken egg cartons plates were used as montages for cocoon spinning (Zannoon and Shadia 1994).

Plant species and preparation of plant extracts

The fresh leaves of mulberry leaves (*Morus alba*) and Basil leaves (*Ocimum basilicum*) were collected from mulberry trees and basil plant, and washed with tap water to remove soil and unwanted dust particles, then the leaves were air dried and then powdered by using mechanical blender and stored in air tight bottles. The *Nigella sativa* seeds were bought from a local herbal market, then powdered and stored as mentioned before. The extracts were prepared by soaking the plant powders in petroleum-ether for 4 days, then the petroleum-ether extracts were filtered by using filter papers. A rotary evaporator apparatus was used to remove the solvent, and the remaining extracts were stored in dark bottles in the refrigerator until use (Harborne 1984).

Isolation of bacteria

Bacterial isolation and identification was carried out in Agricultural Microbiology 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 2003).

Preparation and count of bacterial culture

Bacillus thuringiensis were cultured over night 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 count 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 bacterial spore suspension was made by take 900, 800, 700,600, 500,400,300, 200 and 100 µm from stock solution and complete each one to 1 ml by saline and take 1 ml from stock solution for 100% for making the different concentrations 100%,90%,80%,70%,60%, 50%, 40%, 30%, 20% and 10% (Cockerill et al. 2012).

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) and Meyling (2007). Culture of *Beauveria bassiana* was maintained on Potato Dextrose Agar slants and was sub-cultured in Petri dishes according to Linday (1962). The conidial concentration (1×10^7 conidia /ml) was estimated using Neubauerhemocytometer under a Leitz DialuX20EB microscope (400x magnifications) according to Yeo et al. (2003).

Infecting of silkworm larvae with bacteria and Fungi

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

Experimental design and larval treatment

The newly exuviated fourth instar larvae were separated into 3 groups with 100 larvae/group. One group infected with bacterial spore suspension (50%) of bacterial *Bt* by spraying mulberry leaves and larvae, second group immersed in the fungal spores solution, and the third one immersed in distilled water only (Normal control), Both the first and second groups were divided into four other groups, three of which were treated with the materials used as follows:

Treat.1, 2, 3 - sprayed with 3, 2 and 1% *M. alba* leaves extract.

Treat.4, 5, 6- sprayed with 3, 2 and 1% *Nigella sativa* seeds extract.

Treat.7, 8, 9 - sprayed with 3, 2 and 1% *Ocimum basilicum* leaves extract.

The fourth one used as infested control. All concentrations were applied as bed disinfectant three times during

each of 4th and 5th larval instars by spraying over the larvae and mulberry leaves an hour before morning meal.

Developmental and technological measurements

Different biological characteristics were measured such as, larval mortality (%), larval weight, larval duration, pupal weight, Fresh cocoon weight, shell cocoon weight and cocoon silk ratio was calculated according to Tanaka (1964) formula:

$$\text{Silk ratio(\%)} = \frac{\text{Weight of cocoon shell (g)}}{\text{Weight of fresh cocoon (g)}} \times 1000$$

For technological measurements, five cocoons of each treatment were dried in an oven at 60 °C for 8 h to be reeled individually (Shaaban 1997). The length of reeled silk filament was measured and weighed for each cocoon. The size of the reeled silk filament (denier) was estimated according to Tanaka (1964) formula:

$$\text{Size (dn)} = \frac{\text{Weight of silk filament (g)}}{\text{Length of filament (m)}} \times 9000$$

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA), IBM Costat V.6.311 (2005) Software Means were compared using Duncan's test (≤ 0.05) according to Snedecor and Cochran (1982).

Results

Biological parameters of silkworm larvae treated by some extracts as disinfectant to control artificially infected with *B. thuriangiensis*

As shown in (Table 1) analysis of data revealed that, the differences between means larval weights were highly significant. The highest larval weights recorded 4.084, 4.032, 4.059 and 4.036 g for infected larvae treated with the extracts of 2, 3% basil leaves and 3, 2% black seed as compared to 3.008 g for infected control larvae.

Treating infected larvae by 2, 3% black seed and 3, 2% basil leaves decreased the larval mortality to 10.00, 12.00, 12.00, 12 and 10%, respectively compared to 26.00% mortality in infected control larvae, while the lowest death rate was recorded for the normal control. The differences between means in this parameter are highly significant. Regarding silkworm pupal weight, the highest means (1.266 g) was recorded for infected larvae fed on mulberry leaves treated with 3% basil leaves extract. The normal control gave a higher pupal weight than the most coefficients used treatment. Analysis of data revealed highly significant differences among the means of pupal weights (Table 1).

Table 1 Effect of Mulberry leaves, Black seeds and Basil leaves extracts as disinfectants on some biological and cocoon characters of infected Silkworm, *B. mori* larvae with *B. thuringiensis*

Extracts	Conc. ^a	Larval weight (g)	Larval Mortality %	Pupal weight (g)	Cocoon weight (g)	Cocoon shell wt. (g)	Silk ratio %
Mulberry Leaves	3%	3.736 ^a	16.0 ^a	1.06	1.382 ^a	0.318 ^a	23.13 ^a
	2%	3.563 ^a	16.0 ^a	1.082	1.362 ^a	0.322 ^a	23.57 ^a
	1%	3.785 ^a	16.0 ^a	1.036	1.408 ^a	0.288 ^a	23.53 ^a
Black seed	3%	4.059 ^a	12.0 ^a	1.092	1.336 ^a	0.342 ^a	25.56 ^a
	2%	4.036 ^a	10.0 ^a	1.052	1.394 ^a	0.338 ^a	24.26 ^a
	1%	3.75 ^a	12.66 ^a	1.156	1.404 ^a	0.342 ^a	24.42 ^a
Basil Leaves	3%	4.032 ^a	10.0 ^a	1.266	1.642 ^a	0.354 ^a	21.56 ^a
	2%	4.084 ^a	12.0 ^a	1.114	1.428 ^a	0.342 ^a	23.94 ^a
	1%	3.932 ^a	14.0 ^a	0.940	1.244 ^a	0.296 ^a	23.81 ^a
<i>B. thuringiensis</i> control		3.008 ^a	26.0 ^a	0.954	1.216 ^a	0.26 ^a	21.38 ^a
Control		3.769 ^a	0.66 ^a	1.116	1.348 ^a	0.332 ^a	24.66 ^a
F		11.435	40.141	3.114	15.606	2.888	1.878
LSD _{0.05}		0.254	3.064	0.143	0.076	0.048	3.43
$P \geq 0.05$		0.000**	0.000**	.0031**	0.000**	0.0054 **	0.0665*

^aThe tested concentrations of plant extracts (%)

*,** denote significant and highly significant differences at 0.05, 0.01 levels of probability

Cocoon characters

Feeding silkworm, *B. mori* larvae on mulberry leaves treated with 3, 2% basil leaves extract resulted in the highest cocoon weight recording 1.642 and 1.428 g. On the other hand, treating silkworm, *B. mori* with infected mulberry leaves by bacterial suspension (*B. thuringiensis*) attained the least cocoon weight 1.216 g (Table 1). Other tested extract concentration showed intermediate values. Meanwhile, the normal control gave relatively high values for the weight of fresh cocoons. The differences of means revealed highly significant among concentrations in this parameter.

Statistical analysis of data revealed highly significant differences in cocoon shell weights among the concentrations. Data in (Table 1) indicated that, the highest cocoon shell weights recorded 0.354, 0.342, 0.342 and 0.342 g for infected larvae fed on mulberry leaves treated with the extracts of 3, 2% basil leaves and 3, 1% black seed, respectively. The normal control recorded relatively high values of 0.332 g in comparison with infested control which recorded 0.260 g.

As shown in (Table 1), the maximum silk ratios were detected when infected larvae fed on mulberry leaves treated with 3, control, 2 and 1% black seed extract recording, 25.56, 24.66, 24.26 and 24.42%, respectively. On the contrary, the least silk ratio 21.38% was noticed with control-infected larvae without treatment. The differences between silk ratio means are significant.

Silk filament characters

Concerning silk filament length, all tested concentrations increased the silk filament length with highly

significant differences between means (Table 2). The corresponding lowest silk filament length 775.35 m was recorded for infected control larvae. Moreover, the longest filament length was recorded 1271.50, 1226.50 and 1214.5 m for cocoons resulted from larvae treated by high concentration of mulberry leaves, basil leaves and black seeds, respectively.

Analysis of data revealed highly significant differences among the silk filament weight means (Table 2). The maximum value 0.318 g was noticed for control larvae. All tested treatments increased filament weights comparing with filament weights resulting from cocoons spun by larvae treated with infected mulberry leaves by bacterial suspension.

Data tabulated in Table 2 revealed highly significant differences between silk filament size means. The highest silk filament size 2.627 dn was recorded for control group, while the lowest mean 1.58 dn for infected larvae fed on mulberry leaves sprayed with 1% black seed extract.

Biological parameters of silkworm larvae treated by some extracts as disinfectants to control artificially infected with *B. bassiana*

Analysis of data revealed highly significant differences among the silk filament weight means (Table 2). The maximum value 0.318 g was noticed for control larvae. All tested treatments increased filament weights comparing with filament weights resulting from cocoons spun by larvae treated with infected mulberry leaves by bacterial suspension.

Table 2 Effect of Mulberry leaves, Black seeds and Basil leaves extracts as disinfectants on silk filament characters of infected silkworm, *B. mori* larvae with *B. thuringiensis*

Extracts	Conc. ^a	Silk Filament Length (m)	Silk Filament weight (g)	Silk Filament Size (dn)
Mulberry Leaves	3%	1271.50 ^a	0.300 ^a	2.123 ^a
	2%	1147.75 ^a	0.243 ^a	1.900 ^a
	1%	1092.50 ^a	0.223 ^a	1.830 ^a
Black seed	3%	1214.20 ^a	0.308 ^a	2.280 ^a
	2%	1145.50 ^a	0.233 ^a	1.830 ^a
	1%	1154.50 ^a	0.203 ^a	1.580 ^a
Basil Leaves	3%	1226.50 ^a	0.265 ^a	1.943 ^a
	2%	1149.75 ^a	0.260 ^a	2.033 ^a
	1%	1061.75 ^a	0.238 ^a	2.020 ^a
<i>B. thuringiensis</i> control		775.35 ^a	0.17 ^a	1.973 ^a
Control		1089.50 ^a	0.318 ^a	2.627 ^a
F value		18.411	8.357	6.651
LSD _{0.05}		83.230	0.040	0.320
$P \geq 0.05$		0.0000**	0.0000**	0.0000**

^a The tested concentrations of plant extracts (%)

** denote highly significant differences at 0.05, 0.01 levels of probability

Data tabulated in Table 2 revealed highly significant differences between silk filament size means. The highest silk filament size 2.627 dn was recorded for control group, while the lowest mean 1.58 dn for infected larvae fed on mulberry leaves sprayed with 1% black seed extract. The statistical analysis showed that there is a high significance among the averages showing that the control is better than the used treatments.

As shown in (Table 3), larval weight means ranged between 4.291 to 3.812 g for infested silkworm larvae with

conidial solution of *B. bassiana*, treated with plant extract concentrations under study sprayed increased the larval weight compared to 2.937 for infected control. The differences between larval weight means are highly significant.

Infection of mulberry silkworm with conidial solution of *B. bassiana* increased larval mortality to 27% comparing to 2% mortality for control. All plant concentrations under study decreased the larval mortality especially 1% mulberry leaves and 2, 1% black seed extracts.

Table 3 Effect of Mulberry leaves, Black seeds and Basil leaves extracts as disinfectants on some biological and cocoon characters of infected Silkworm, *B. mori* larvae with *B. bassiana*

Treatment	Conc. ^a	Larval weight (g)	Larval Mortality (g)	Pupal weight (g)	Cocoon weight (g)	Cocoon shell wt. (g)	Silk ratio %
Mulberry Leaves	3%	4.028 ^a	10.67 ^a	1.194 ^a	1.552 ^a	0.358 ^a	23.058 ^a
	2%	4.165 ^a	9.33 ^a	1.120 ^a	1.462 ^a	0.334 ^a	22.920 ^a
	1%	3.995 ^a	8.00 ^a	1.038 ^a	1.370 ^a	0.324 ^a	23.652 ^a
Black seed	3%	4.117 ^a	11.33 ^a	1.408 ^a	1.776 ^a	0.362 ^a	21.862 ^a
	2%	4.03 ^a	8.00 ^a	1.208 ^a	1.556 ^a	0.340 ^a	21.896 ^a
	1%	3.812 ^a	8.66 ^a	1.150 ^a	1.464 ^a	0.310 ^a	21.228 ^a
Basil Leaves	3%	4.291 ^a	11.33 ^a	1.580 ^a	1.988 ^a	0.382 ^a	19.258 ^a
	2%	4.055 ^a	10.67 ^a	1.300 ^a	1.652 ^a	0.350 ^a	21.150 ^a
	1%	3.924 ^a	9.33 ^a	1.128 ^a	1.394 ^a	0.314 ^a	22.566 ^a
<i>B. bassiana</i> infected control		2.937 ^a	27.00 ^a	0.980 ^a	1.274 ^a	0.286 ^a	22.440 ^a
Control		3.769 ^a	2.00 ^a	1.116 ^a	1.348 ^a	0.332 ^a	24.660 ^a
F value		12.664	28.081	21.115	39.134	2.769	1.175
LSD _{0.05}		0.285	3.451	0.102	0.088	0.049	ns
$P \geq 0.05$		0.0000**	0.0000**	0.0000**	0.0000**	.0073**	0.3287

^a The tested concentrations of plant extracts (%)

ns and ** denote not significant and highly significant differences at 0.05, 0.01 levels of probability

Data presented in (Table 3) showed that, *B. mori* pupal weight recorded the highest means (1.580 and 1.408 g), when infected larvae treated 3% basil leaves and 3% black seeds extracts. On the contrary, the least pupal weight mean (0.980 g) was resulted from *B. bassiana* infected control.

Cocoon characters

Statistical analysis of data in (Table 3) revealed highly significant differences in fresh cocoon weights among different treatment under study. It was found that, spraying infected silkworm, *B. mori* larvae with mulberry leaves, basil leaves and black seed extracts at 3, 2 and 1% concentrations increased fresh cocoon weights as compared to control. On the other hand, *B. bassiana* infected control recorded the least cocoon weight.

Regarding cocoon shell weight, infection of mulberry silkworm with conidial solution of *B. bassiana* decreased cocoon shell weight to 0.286 g as compared to 0.332 g for control. Spraying infected larvae by 3 and 2% of mulberry leaves, black seed and basil leaves extracts increased cocoon shell weight (Table 3). Analysis of data, revealed highly significant differences among means. With respect to silk ratio, analysis of data in (Table 3) revealed that, there no differences among means are non-significant. Control larvae occupied the first rank in silk ratio recording 24.660%, followed by treating infected larvae with all concentrations of mulberry leaves extract.

Silk filament characters

Concerning silk filament length, it was found that infection of *B. mori* larvae with *B. bassiana* conidial solution decreased silk filament length to 691.50 m comparing to 1089.50 m for control. Treatment of infected larvae with investigated plant extracts as disinfectants at 3 and 2% increased silk filament length as shown in Table 4. Application of all tested concentrations of mulberry leaves, black seeds and basil leaves extracts on infected *B. mori* larvae with *B. bassiana* conidial solution increased the silk filament weight as compared to infected not treated group (Table 4). With regard of silk filament size, it was observed that control group gave the highest value (2.627 dn), meanwhile, 3% mulberry leaves extract showed the least value (1.755 dn). Other tested concentrations gave intermediate values. Analysis of data in Table 4, revealed highly significant differences among the means for the above mentioned silk filament traits.

Discussion

Disinfection and maintenance of hygiene during rearing are the essential factors for preventing of silkworm diseases. Disease incidence occur in all larval instars but more commonly in the 4th and 5th instars and cause

Table 4 Effect of Mulberry leaves, Black seed and Basil leaves extracts as disinfectants on silk filament characters of infected Silkworm, *B. mori* larvae with *B. bassiana*

Extracts	Conc. ^a	Silk Length (m)	Silk weight (g)	Silk size (dn)
Mulberry Leaves	3%	1153.00 ^a	0.225 ^a	1.755 ^a
	2%	1103.75 ^a	0.223 ^a	1.823 ^a
	1%	993.750 ^a	0.203 ^a	1.840 ^a
Black seed	3%	1286.40 ^a	0.290 ^a	2.070 ^a
	2%	1158.45 ^a	0.253 ^a	1.963 ^a
	1%	1012.25 ^d	0.240 ^a	2.138 ^a
Basil Leaves	3%	1106.40 ^a	0.315 ^a	2.550 ^a
	2%	1085.00 ^a	0.275 ^a	2.273 ^a
	1%	958.40 ^a	0.280 ^a	2.430 ^a
<i>B. bassiana</i> infected control		691.50 ^a	0.160 ^a	2.080 ^a
Control		1089.50 ^a	0.318 ^a	2.627 ^a
F value		26.028	7.804	5.491
LSD _{0.05}		98.2	0.05	0.34
P ≥ 0.05		0.0000**	0.000**	0.000**

^a The tested concentrations of plant extracts (%)

** denote highly significant differences at 0.05, 0.01 levels of probability

cocoon crop losses (Khurad et al. 2004). The incidence of diseases at the time of silkworm rearing severely decreased the production of silk. *Bacillus* has been identified as one of the most important pathogenic organism involve in flacherie (Steinhuaus 1949).

According to the obtained results, using the plant extracts to the infected mulberry silkworm, *B. mori* with bacterial suspension (*B. thuringensis*) indirectly increased larval weight and decreased the larval mortality of both 4th and 5th instars because of eliminating the chance of larval infection. Our results indicated that, basil leaves and black seed extracts proved to give the highest results for biological and cocoon characters. The antimicrobial activity of *O. basilicum* (basil) is due to its phenolic acids and aromatic compounds Gutierrez et al. (2008). Lachowicz et al. (1998) cleared that, the essential oils from *Ocimum* species were predominantly associated with the main constituent's linalool and methyl chavicol which responsible for antibactericidal effect on gram-positive and gram-negative bacteria. So it can be used as disinfectant for infected silkworm and to increase the cocoon production. The same results were obtained by Hassan and Saad (2012) who found that, larval weight increased significantly when treated with germ wheat oil.

The obtained results of the investigated plant extracts showed that, plant extracts at different concentrations showed not only antibacterial activity against the bacteria (*B. thuriengensis*) that infected the mulberry silkworm but also showed improvement of the economic traits. Herbal extracts show antimicrobial activity against some

pathogenic microorganisms including Gram positive bacteria such as *S. aureus* and *B. subtilis* and Gram-negative bacteria such as *E. coli* (Whitt and Salyers 2002; Sivakumar et al. 2012). Similar observation was indicated by Mohanta et al. (2013) who stated that, ethanol extracts of *C. zeylanicum*, *C. longa*, *Z. officinale* plants showed a strong antibacterial activity against pathogens isolated from diseased silkworm with bacterial Flacherie.

Sujatha and Sampath (2015) stated that, clove oil was active against *Bacillus cereus* and *Proteus vulgaris* and could be used for controlling the flacherie disease, which infected mulberry silkworm, *B. mori*. It found that, clove oil increased significantly cocoon parameters of silkworm as compared to control. Similarly, chloroform leaf extract of *Aegle marmelos* and bark extracts of *Thuja orientalis* were affecting in managing *B. thuringiensis* and *S. aureus* infecting *B. mori* (Manimegalai et al. 2010). These results are in partial accordance with Shyam et al. (2017) who treated infected silkworm with the *Trichosanthes cucumerina* seeds and leaves extract and found that, seed extract of *T. cucumerina* at T1 (T1 = 1:1 = plant: water) recorded maximum cocoon weight, pupal weight, shell weight, silk ratio percentage and filament length. Moreover, El-Sayed (1999) stated that, the mixture of honey and black seeds increased silk production and the number of deposited eggs/female and gave heaviest weight of larvae, pupae and increased the total protein of the silk gland.

Obtained results indicated that, all concentrations of the plants under study enhanced the silk filament length, weight and size in comparing with silk filaments resulting from cocoons spun by larvae treated with infected mulberry leaves with bacterial suspension. Similar results were reported by Elyamani and Zannoon (2017) who studied the effect of fortification of mulberry leaves with *Cichorium intybus* L. aqueous extract on some biological and technological characters of silkworm, *B. mori* and found that, all tested concentrations increased silk filament length as compared to control.

Muscardine infection was reported higher during winter season, less during rainy season and it may be due to low temperature with high humidity in silkworm rearing environment, which work as a pre-requisite for outbreak of the fungal disease and efficacy of the bed disinfectants (Chandrasekharan 2009). In Egypt, the incidence of muscardine infection is very rare because most silkworm rearing occur during spring and autumn seasons, where the weather is moderate in temperature and humidity.

Obtained results indicated that, artificial infection of mulberry silkworm with conidial solution of *B. bassiana* increased larval mortality and decreased larval weight of silkworm, *B. mori*. The cocoons spun by diseased larvae with *B. bassiana* became weak and have light weight and

silk ratio, also the reeled silk filaments were short in lengths and had small sizes and weights.

Study the efficacy of petroleum-ether extracts of *M. alba*, *O. basilicum* leaves and *N. sativa* seeds as disinfectants against *B. bassiana* proved that, those plant extracts were effective and caused improvement in most traits under study. Similar results were recorded by Rehab et al. (2016) who obtained highest larval weight and Cocoon weight after application of LC (mix of *Lawson inainermis* 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). These results were agree with the finding of Padma and Ramani (2014) who stated that, ethanolic leaf extract of *O. sanctum* was effective in controlling white muscardine disease caused by *B. bassiana* and increased the cocoon weight, pupal weight, shell weight and shell ratio. Recently several reports have come on antimicrobial activity of plant extracts against bacteria and fungi (Mohanan et al. 2007; Chavan et al. 2013). 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 et al. 2018). Moreover, Soumya (2011) proved that, the two botanicals (*Eucalyptus* hybrid and *Moriga olifera*) when dusted at 100% every day on silkworm inoculated with stock solution of fungal spore suspension enhanced larval weight, cocoon weight, shell weight, pupal weight, shell ratio, silk filament length, denier and reduced larval mortality compared to other treatments.

Most of the biological activity of *Nigella sativa* seeds showed by the oil constituents like, Thymoquinone (El-Tahir and Bakeet 2006). The antifungal activity of Thymoquinone from *Nigella sativa* had evaluated by the standard agar plate method (Aljabre et al. 2005).

Conclusions

Tested concentrations of *Morus alba*, *Ocimum basilicum* leaves extracts and *Nigella sativa* seeds extract have suppressed the bacterial and fungal disease spread when used three times through both 4th and 5th larval instars. Moreover, the biological characters and technological traits enhanced indirectly by using of these extracts, so it can be used in sericulture for improving cocoon crop production, i.e., quality and quantity.

Abbreviations

Dn: Denier (silk filament size measuring unit); FL: Formaldehyde and lime powder 0.25: 0.75; LC: Mix of *Lawson inainermis* and *Curcuma longa* powders Juvenile hormones; NaCl: Sodium chloride

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Authors' contributions

MSIS carried out the breeding and physiological studies, participated in the design of the study, performed the statistical analysis and helped to revise the manuscript. EMEY participated in the sequence alignment and drafted the manuscript, and helped to revise the manuscript. WMMH conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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