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Influence of some natural products of *Moringa oleifera* (L.) on some biochemical and economical characters of infected mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

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Abstract

Bacterial diseases of silkworm, *Bombyx mori* cause serious problem during silkworm rearing. They cause quantitative and qualitative reduction of silk production. Use of botanical materials having anti-microbial property with no side effects is an alternative way for controlling silkworm diseases. This study aims to shed light on the role of some natural products of *Moringa oleifera* (seed oil, leaves extract, root powder and honey) with different concentrations in treatment of disease silkworm with bacterial infection, by studying their effects on some protein enzymes Alanine transaminase (ALT), Aspartate transaminase (AST) and protease enzymes, cocoon parameters and silk filament characters of *Bombyx mori*. At the beginning of the 5th larval instar, larvae were fed on fresh mulberry leaves supplemented with different concentrations of extracts (three diets/day, five times during the 5th larval instar). A significant increase of biochemical aspects (protein transaminases and protease enzyme) in all treatments was observed in comparison with control. Root extracts with concentrations (1% & 2%) and 1.5% of seed oil were the best. Similarly, the cocoon characters (cocoon and shell weights) and silk characters (filament length, weight and size) enhanced significantly in high concentrations. Root and seed oil extracts exhibited the highest significant effect, while no significant differences were noticed among shell weight means. Extracts of the different parts of *M. oleifera* have a protective and therapeutic execution against bacterial infection of *B. mori*. Furthermore, a highly significant difference was noticed with root extract using followed by the seed oil treatment with higher concentrations among other treatments.

Keywords: Bacterial diseases, botanical extracts, *Moringa oleifera*, *Bombyx mori*.

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Introduction

The mulberry silkworm, *Bombyx mori* L., (Lepidoptera: Bombycidae) is one of the most economic important insects. This importance developed from the ability of silkworm to secrete the natural silk filament from its silk gland. Silk production is only about 0.2 % of the total textile fiber production in the world which represents a slow production increase. Recently a considerable attention has been given to improve rearing technology and consequently increasing natural raw silk production. However, this production may be suddenly falls due to many technical and non-technical problems. Silkworm diseases are considered as one of the major technical problem; Bacterial diseases (Flacherie) are usually only secondary to virus diseases. Several bacteria cause Septicaemia and toxaemia (*Bacillus*, *Streptococcus*, *Staphylococcus* and *Escherichia coli*) cause softening and putrefaction of the dead worms. Tolerance to all the agents is another theory to decreasing the incidence rate of those diseases. Finding natural and eco-friendly plant products that prevent or treat these diseases could be an alternative treatment process (Kumar et al., 2009; Mesbah et al., 2000; Mahesha et al., 1999; Parra, 1991). Use of herbal plants in medicine which having anti-microbial property, non-toxic, biodegradable and non pollutant for controlling diseases of silkworm rearing. Plant extracts contain variety of components that can either inhibit the growth of the microorganisms or eradicate them were used by many authors (Abalaka et al., 2009; Nigam, 1982). *Moringa oleifera* L. belongs to family Moringaceae. It originally located in Asia then spread in many parts of Africa. This family contains around 13 species relocated from tropical to subtropical regions and ranging in size from little herbs to huge trees and recently grows successfully in Egypt. The importance of this plant is due to its multiple uses and benefits to agriculture and industry and the all parts of *Moringa* plant are used for medicinal and other purposes (Barakat & Ghazal, 2016; Janick & Paull, 2008; Anwar et al., 2007; Price, 2000). *M. oleifera* contains many essential nutrients, vitamins, minerals, amino acids, beta-carotene, omega 3 and 6 fatty acids, also it consists of antioxidants, anti-inflammatory, anti-spasmotic, anti-hypertensive, anti-tumour, anti-pyretic, anti-ulcer, cholesterol lowering and anti-diabetic nutrients (Sharma et al., 2012; Paliwal et al., 2011; Kasolo et al., 2010; Hsu et al., 2006; Fahey, 2005). Physicochemical properties of *Moringa* seeds oil proved that this oil bodes rewarding potential application in nutrition aspects. The higher content of unsaturated fatty acids may present a healthy influence of *Moringa* seeds oil in terms of nutrition (Barakat & Ghazal, 2016). It has good quantity of oleic acid (57%) and omega 3 (13.28%) and rich in natural antioxidants that are scavenging of free radicals in the body due to the presence of tocopherols, phenolics and carotenoids (Khattab & Shakak, 2012). Leaves of *Moringa* species are rich in various phytochemical components like carotenoids, amino acids, sterols, glycosides, alkaloids, flavonoids, moringine, moringinine, phytoestrogens caffeoylquinic acids and phenolics, and it works as an effective source of natural antioxidants due to the presence of flavonoids, ascorbic acid, carotenoids, and phenolics (Anwar et al., 2007; Siddhuraju & Becker, 2003; Dillard & German, 2000). The present study aims to evaluate the role of some natural products of *Moringa* plant (seed oil, leaves extract, root powder and *Moringa* honey bee) with different

concentrations in treatment of bacterial infected mulberry silkworm, *B. mori*. Also, to select the effective natural part product through studying their effects on the activity of some protein enzymes, cocoon and filament characters.

Materials and methods

The present study was carried out in the laboratory of Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Center, Cairo, Egypt. Mulberry silkworm, *B. mori* eggs ($G_2 \times V_2 \times H_8 \times$ KK hybrid) were obtained from the Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

Silkworm rearing technique: Rearing of silkworm was carried out in laboratory under the hygro-thermic conditions 28 ± 1°C and 75 ± 5% RH, according to the technique of Krishnaswami (1978). The larval bed was cleaned daily. Cleaning net was used for removing the remained dried food and feces. The newly hatched larvae were fed on fresh clean mulberry leaves until the beginning of the 5^{th} instar. The 5^{th} instar larvae were used in the present study. The source of *Moringa* seed oil, leaves extract and root powder were kindly prepared and obtained from Egyptian Scientific Society of *Moringa* (ESSM), National Research Center, Cairo, Egypt. *Moringa* bee honey was obtained from Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. The collected plant parts (seeds, leaves and roots) of *Moringa* were washed under tap water followed by distilled water and dried under shade. Dried samples were powdered in mixer/grinder. Powder of leaves and roots was mixed individually with distilled water in a ratio of 1:1 (w/v) and left overnight to allow the constituents to get dissolved in water, then filtered through muslin cloth and 100% plant extract solution was prepared according to El–Mohamedy and Abdalla (2014). Extraction of seeds oil was prepared according to Harvey and John (1898) protocol. Each was used with three concentrations. 5 ml of *Moringa* oil dissolved in 1 L. distilled water to prepare a concentration of 0.5% by adding Tween 80 to disperse the oil in the solution and 10 ml of *Moringa* oil to prepare a concentration of 1%; and so the remaining concentration to 1.5%. By the same way the other concentrations of *Moringa* leaves extract (0.5%, 1% & 1.5%), *Moringa* root extract (0.5%, 1% & 2%) and *Moringa* honey (1%, 2% & 4%) were prepared without tween 80.

Schedule of application: Larvae were divided into four groups; each group was fed on mulberry leaves treated with (*Moringa* seeds oil, *Moringa* leaves extract, *Moringa* honey and *Moringa* root powder). Each group was divided into three subgroups representing three concentrations with 3 replicates each (100 larvae for each replicate). Mulberry leaves were washed and let to dry. Fresh mulberry leaves were dipped in each concentration for 5 minutes and left to dry then offered to larvae (three diets/day, five times during the larval instar) to obtain the full growth. Fifth group (control) fed on leaves was treated only with distilled water. Chicken egg cartons plates were used as montages for cocoon spinning as described by

(Zannoon & Shadia, 1994).

Infecting of silkworm larvae with bacteria: Bacterial pathogens were collected and isolated from diseased larvae (Aneja, 2003). After bacterial culture prepared using Luria agar medium (Suparna et al., 2011), *B. mori* artificially infected by spraying mulberry leaves with the concentration (15 ppm) of bacterial flacherrie (*Streptococcus pneumoniae*) then fed to larvae one time on the 2nd day of the 5th instar larvae.

The biochemical characters: Samples of haemolymph were collected at the 7th day of the 5th larval instar made by removing one of the thoracic legs of the larvae and bending the body to expose the sternum at the position of the removed leg. This ensured proper drainage of the haemolymph, and avoided any risk of internal organs to be destructed. The haemolymph of each treatment was collected in eppendorf tubes 1.5 ml with small crystal of phenyl thiourea (PTU) to prevent melanization of sample and prepared for biochemical assays according to Mahmoud (1988) protocol. The supernatant was immediately assayed colorimetrically to determine Aspartate transaminase (AST), Alanine transaminase (ALT) activities according to the method of Reitman and Frankel (1957). The proteolytic enzyme activity was determined by the casein digestion method described by (Ishaaya et al., 1971).

The economical characters: The resulted cocoons were collected after 7 days from the beginning of spinning for studying the economical characters. Half number of the resulted cocoons of each replicate was used for determining the cocoon characters (cocoon weight, shell weight). The other half was dried at 80 ºC in oven and used to study the filament characters (filament length, weight and size).

Statistical Analysis: Obtained data was subjected to analysis of variance (ANOVA) as one way to find the differences among different treatments. Statistical analysis was conducted using Proc ANOVA in SAS (SAS Institute, 1988). Means separation was conducted using Duncan Multiple Range Test in the same program. Later factorial analysis was conducted to elucidate the significance among different materials and tested concentrations using the same procedures.

Results and Discussion

Biochemical aspects: The biochemical aspects represent one of the most important aspects in silk production. Proteins constitute the major working force for all forms of biological work.

Alanine transaminase (ALT): As represented in Table (1a), data exhibited a significant increase in ALT means of larvae fed on mulberry leaves treated with different concentrations of *Moringa* products. The root extract concentration (1 and 2%) showed the highest means (82.18, 87.72 µg pyruvate/min/ml) respectively, followed by the concentration of seed oil 1.5% (63.71µg pyruvate/min/ml). Also the concentration of *Moringa* honey 4% recorded the higher ALT value (61.87µg pyruvate/min/ml), comparing to the control group (27.70 µg pyruvate

/min/ml). Data represented in Table (2a) indicated that, *Moringa* root extract exhibited significantly the best result among *Moringa* products (73.56 µg pyruvate/min/ml), followed by seed oil (52.94 µg pyruvate/min/ml).

Aspartate transaminase (AST): Table (1a) showed that a significant increase in AST values with different concentrations compared with control (70.09 µg oxaloacetate/min/ml), especially the root extract (2%) exhibited (148.59µg oxaloacetate/min/ml) followed by the 2nd concentration (1%) of the same treatment (132.70 µg oxaloacetate/min/ml), then the 3rd concentration of seed oil (1.5%) followed by the 3rd concentration (4%) of *Moringa* honey (122.42, 104.66 µg oxaloacetate/min/ml) respectively. As shown in Table (2a), a significant increase in AST values of diseased larvae with mulberry leaves supplemented with *Moringa* root extract (125.54 µg oxaloacetate/min/ml), followed by seed oil of *Moringa* treatment (106.22 µg oxaloacetate/min/ml). At the same time, AST means increased significantly with concentrations increasing. According to the obtained results, the significant increase in protein enzymes activities may be attributed to the useful effect of constituents of botanical extracts on the haemolymph protein content of infected silkworm, *B. mori*. These findings are close to those of Yousef (2014), revealed that, the improvement of silk production depending on amines transfer mechanism involved in the uptake of used compounds constituted in mulberry leaves by the body tissues and silk glands, resulted in the subsequent promotion of silk protein synthesis. By the same way, Arora et al. (2013) stated that the various extracts of Moringa's morphological parts such as seeds, steam park, leaves, root bark possess antimicrobial potentiality. Fahey (2005) stated that, the chemical compounds which isolated from the plant *M. oleifera* contain numerous antibacterial compounds such as, glucosinolates, rhamanose, pterygospermin, and isothiocyanates. Gaceres et al. (1992) and Ezeamuzie et al. (1996) reported that, the root extracts of *Moringa oleifera* possess anti-inflammatory activity, so, it has been used for treatment of a diseases number. Similarly, Khattab and Shakak (2012) and Barakat and Ghazal (2016) showed that, the protein content of *Moringa* seed oil was 41.13% and the amino acids content demonstrated a high potential application of seed extracts in nutrition being the highly nutrition value of its protein.

Protease: According to the results obtained in Table (1a), the 3rd concentration (2%) of root extract followed by the 2nd concentration (1%) exhibited significantly the highest means of protease enzyme activity (194.71, 177.18 O.D. units $x10^3$/min/ml), respectively compared to the control group (99.72 O.D. units $x10^3$/min/ml). At the same way, the 3rd concentration (1.5%) of seed oil and the 3rd concentration (4%) of honey also showed significant increase of protease values (152.64 and 149.66 O.D. units $x10^3$ /min/ml), respectively. On the other hand, as shown in Table (2a), a highly significant difference was noticed with *Moringa* root extract treatment (168.40 O.D. units $x10^3$/min/ml), followed by the seed oil treatment (138.65 O.D. units $x10^3$/min/ml). These results are

confirmed by Prajapati et al. (2003) finding. Who described the presence of the essential amino acids - arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine, valine among the biochemical constituents of *Moringa oleifera* which also consists of Carotene, nicotinic acid, ascorbic acid, ascorbic acid oxidase sulphur, a prolamin. As the same trend, roots have been found to possess several distinct pharmacological properties (Goyal et al., 2007). Similarly, Haristory et al. (2005) reported that, the isothiocyanate structure and its predecessor, glucosinolate, as primary constituents from *M. oleifera* seed extracts are grand antibacterial agents. Also, protease regulates the fate, localization, and activity of many proteins, modulate protein-protein interactions and create new bioactive molecules and influence immunity and apoptosis (Turk, 2006), it represents an important tool of the biotechnological industry because of its usefulness as biochemical reagent or in the manufacture of numerous products (Saeki et al., 2007). Generally, the significant increases of the biochemical aspects (protein transaminases and protease enzyme) of the present study are in good agreement with previous work demonstrating that, the natural disinfectants may stimulate the enzymes activities which influence the biochemical contents of the haemolymph of the silkworm, *B. mori*; also, increased the amount of haemolymph protein of larval instars as suggested by El-Sayed et al. (1990).

Table 1a: Effect of different treatment concentrations of *Moringa* on the biochemical characters of diseased mulberry silkworm, *B. mori*.

Treatments	Conc. (%)	ALT	AST	Protease
Moringa seed oil	0.5	42.48 ^{cde}	95.32 ^{cde}	123.99 ^{bc}
	1	52.63 ^{cde}	100.93 ^{cde}	139.32 ^{bc}
	1.5	63.71^{abc}	122.42 ^{abc}	152.64 ^{abc}
Moringa leaves extract	0.5	60.94 ^{bcd}	102.80 ^{bcde}	149.44 ^{abc}
	1	34.17 ^{e}	74.76 ^{de}	106.72 ^{c}
	1.5	37.04 ^{de}	81.30 ^{de}	114.38 ^{c}
Moringa honey	1	32.32 ^{e}	74.76 ^{de}	104.52 ^{c}
	2	34.17 ^{e}	78.50 ^{de}	111.78 ^{c}
	4	61.87 ^{bcd}	104.66 ^{bcd}	149.66 ^{abc}
Moringa root extract	0.5	50.79 ^{cde}	95.32 ^{cde}	133.32 ^{bc}
	1	82.18 ^{ab}	132.70 ^{ab}	177.18 ^{ab}
	2	87.72 ^{a}	148.59 ^{a}	194.71 ^{a}
Control		27.70 ^{e}	70.09 ^{e}	99.72 ^{c}
P value		0.0001	0.0001	0.01
L.S.D.		22.91	28.37	49.17

Means in the same column not followed by the same letter are significantly different ($P \leq 0.05$).

Table 1b: Effect of different treatment concentrations of *Moringa* on the economical characters of diseased mulberry silkworm, *B. mori*.

Treatments	Conc. (%)	Cocoon weight(g)	Shell weight(g)	Filament length(m)	Filament weight(g)	Filament size(dn)
Moringa seed oil	0.5	0.70 ^{abc}	0.11 ^{ab}	385.00 ^{bcd}	0.11 ^{bcd}	2.53 ^{bc}
	1	0.71 ^{abc}	0.09 ^{ab}	452.00 ^{abcd}	0.12 ^{abc}	2.33 ^{bcd}
	1.5	0.79 ^{ab}	0.13 ^{ab}	494.67 ^{ab}	0.13 ^{abc}	2.39 ^{bcd}
Moringa leave extract	0.5	0.73 ^{abc}	0.07 ^{b}	462.67 ^{abcd}	0.11 ^{bcd}	2.14 ^{cd}
	1	0.52 ^{bc}	0.09 ^{ab}	287.67 ^{cde}	0.07 ^{de}	2.07 ^{cd}
	1.5	0.69 ^{abc}	0.13 ^{ab}	382.33 ^{bcd}	0.10 ^{cd}	2.35 ^{bcd}
Moringa honey	1	0.52 ^{bc}	0.08 ^{ab}	276.00 ^{de}	0.10 ^{cd}	3.17 ^{a}
	2	0.65 ^{abc}	0.10 ^{ab}	351.00 ^{bcde}	0.07 ^{de}	1.70 ^{d}
	4	0.76 ^{abc}	0.12 ^{ab}	469.00 ^{abc}	0.12 ^{abc}	2.27 ^{bcd}
Moringa root extract	0.5	0.70 ^{abc}	0.13 ^{ab}	431.00 ^{bcd}	0.11 ^{bcd}	2.46 ^{bc}
	1	0.82 ^{a}	0.14 ^{a}	506.00 ^{ab}	0.15 ^{ab}	2.62 ^{ab}
	2	0.92 ^{a}	0.13 ^{ab}	626.00 ^{a}	0.16 ^{a}	2.31 ^{bcd}
Control		0.49 ^{c}	0.11 ^{ab}	193.00 ^{e}	0.05 ^{e}	2.17 ^{cd}
P value		0.0328	0.2504	0.0013	0.0002	0.0001
L.S.D.		0.2367	-	163.8	0.03986	0.626

Means in the same column not followed by the same letter are significantly different (P≤ 0.05).

Economic aspects (Cocoon indices): Cocoon indices represented the most important economic parameters in silk industry. Fresh cocoon weight, cocoon shell weight and silk ratio were recorded in Tables (2a & 2b). Fresh cocoon weight (g): Results in Table (1b) indicated that all different concentrations of used treatments recorded significant increases in cocoon weights, compared with control group (0.49 g). Among different concentrations, (1% & 2%) of *Moringa* root extract exhibited the highest weights (0.82& 0.92 g) followed by the concentration 1.5% of *Moringa* oil (0.79 g). By the same way, data tabulated in Table (2b) cleared that, a significant increase in fresh cocoon weights was noticed among all treatments; root extract recorded the highest value (0.82 g) followed by seed oil (0.73g). Cocoon shell weight (g): Data represented in Tables (1b & 2b) showed that, no significant differences in shell weights were noticed among investigated treatments and with their different concentrations. Increasing of cocoon parameters in the present study are due to the stimulation after using different *Moringa* extracts for treating the 5th instar larvae. These findings are in confirmation with those of Rajeswari and Isaiarasu (2004) who found that, the dietary supplementation of leaf, flower and pod extracts of *M. oleifera* (1% w/v) trigger varied responses in the 5th instar larvae of *Bombyx mori;* as a result of this supplementation. The means of larval weight and the weight and size of cocoon were increased significantly. Patil et al. (2005) reported that *Parthenium* root extract motivated silkworms to feed more, resulting in the increase of larval, cocoon and pupal weight. Furthermore, Mbikay (2012) noticed that, studies with the *Moringa* plant have recommended its efficacy in treating inflammation and these properties of its phytochemicals due to the presence of flavonols, and phenolic acids which related to the anti-inflammatory, anti-oxidants and anti-bacterial activities. Karagiorgou et al. (2016) cleared that, the polyphenolic composition of *M. oleifera* roots remains

largely unexamined and the results indicated that water extracts were the richest in total polyphenols, exhibiting strong antioxidant activity.

Table 2a: Effect of different treatments and different concentrations of *Moringa* on the biochemical characters of diseased mulberry silkworm, *B. mori*.

Factor	Level	ALT	AST	Protease
Treatments	M. seed oil	52.94 [b]	106.22 [ab]	138.65 [ab]
	M. leave extract	44.05 [b]	86.29 [b]	123.51 [b]
	M. honey	42.79 [b]	85.97 [b]	121.99 [b]
	M. root extract	73.56 [a]	125.54 [a]	168.40 [a]
P value		0.0024	0.0006	0.0173
L.S.D.		16.73	19.66	31.29
Concentrations	C1	46.63 [b]	92.05 [b]	127.82 [a]
	C2	50.79 [ab]	96.72 [b]	133.75 [a]
	C3	62.59 [a]	114.24 [a]	152.85 [a]
P value		0.0819	0.0303	0.1608
L.S.D.		-	17.03	-

Means in the same column not followed by the same letter are significantly different (P≤ 0.05).

Table 2b: Effect of different treatments and different concentrations of *Moringa* on the economical characters of diseased mulberry silkworm, *B. mori*.

Factor	Level	Cocoon weight (g)	Shell weight (g)	Filament length (m)	Filament weight (g)	Filament size (dn)
Treatments	M. seed oil	0.73 [ab]	0.11 [a]	443.89 [ab]	0.12 [b]	2.42 [ab]
	M. leave extract	0.65 [b]	0.10 [b]	377.56 [b]	0.09 [c]	2.19 [b]
	M. honey	0.64 [b]	0.10 [a]	365.33 [b]	0.10 [c]	2.38 [b]
	M. root extract	0.82 [a]	0.13 [a]	521.00 [a]	0.14 [a]	2.46 [a]
P value		0.0396	0.1021	0.0168	0.0004	0.0026
L.S.D.		0.1342	-	103.6	0.0229	0.425
Concentrations	C1	0.66 [b]	0.10 [a]	388.67 [b]	0.11 [b]	2.58 [a]
	C2	0.68 [ab]	0.11 [a]	399.17 [b]	0.10 [b]	2.18 [b]
	C3	0.79 [a]	0.13 [a]	493.00 [a]	0.13 [a]	2.34 [b]
P value		0.0607	0.0734	0.0458	0.0042	0.0007
L.S.D.		-	-	89.68	0.199	0.368

Means in the same column not followed by the same letter are significantly different (P≤ 0.05).

Reelable filament characters: Silk filament characters are economically very important. A significant increase was recorded in silk filament characters as a result of feeding diseased silkworm during rearing on mulberry leaves supplemented with different concentrations of natural *Moringa* extracts. Filament length (m): According to the results obtained in Table (1b), a significant increase in filament lengths for all treatments with different concentrations was exhibited; the 3rd concentration (2%) of root extract showed the highest filament length (626 m), followed by the 2nd concentration of it (1%) and the concentration (1.5%) of seed oil (506 & 494.67 m) respectively, followed by the 3rd concentration (4%) of honey (469 m), while the control group recorded the lowest length (193 m). By the same way, data in Table (2b) revealed that *Moringa* root extract exhibited significantly the highest silk filament lengths (521 m) among other treatments, followed by *Moringa* oil

(443.89 m) with increased concentrations. Filament weight (g): means of silk filament weights represented in Table (1b), showed that the highest weights significantly recorded by 2% followed by 1% of *Moringa* root extract (0.16 & 0.15g) respectively, followed by the concentrations 1%, 1.5% of seed oil and 4% of *Moringa* honey (0.12, 0.13 and 0.12g) respectively. While the control recorded (0.05g). Data in table (2b), revealed that root extract treatment significantly exhibited the highest means values in filament weight (0.14g) followed by seed oil treatment (0.12g) with increased concentrations. Filament size (dn): The results as shown in Table (2b) indicated that, root extract treatment was effective significantly (2.46 dn) followed by seed oil treatment (2.42 dn). It might be due to bioactive compounds which have growth promoting and nutritive nature of this plant. These results are in line with Murugan et al. (1998), noticed a strong correlation between the growth of silkworm and the silk production after the treatment of plant extracts. And with Karthikairaj et al. (2014), proved that aqueous and alcoholic extracts of Ocimum, Acalypha, and Leucas can be exploited to control the microbial pathogens during silkworm rearing resulting improved silk yield. This has been further confirmed by Fakurazi et al. (2012) and Anwar et al. (2007) reported that, the various parts (roots, leaves, gum, flowers and seed infusion) of *Moringa oleifera* contain nitrile, mustard oil glycosides and thiocarbamate glycosides which represent important bioactive constituents and have wide medicinal applicability. Conclusively, Plant derived medicines have been part of our conventional health care in most parts of the world and now there is an increasing interest in using plants as the sources of agents to fight microbial diseases (Sandhya et al., 2006). So, the findings of this study proved that *Moringa oliefera* extracts (seeds, leaves, honey and roots) has a protective and therapeutic role in body cells of silkworm *B. mori* against bacterial infections; especially, the root and seed oil extracts with increasing concentrations compared with the infected control group.

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References

Abalaka ME, Olonitola OS, Onaolapo JA, 2009. Evaluation of acute toxicity of *Momordica charantia* extract, using wistar rats to determine safety level and usefulness of the plant ethnochemotheraphy. International Journal of Applied Science **3**: 1–6.

Aneja KR, 2003. Experiments in microbiology, Plant pathology and biotechnology. *New Age International (P) Limited Publeshers*, 4th Edition, pp. 376.

Anwar F, Latif S, Ashraf M, Gilan AH, 2007. *Moringa oleifera*: A food plant with multiple medicianl uses. Phytotherapy Research **21**(1): 17–25.

Arora DS, Onsare JM, Kuar H, 2013. Bioprospecting of *Moringa* (Moringaceae): microbiological perspective. Journal of Pharmacognosy and Phytochemistry **1**: 193–215.

Barakat H, Ghazal GA, 2016. Physicochemical properties of *Moringa oleifera* seeds and their edible oil cultivated at different regions in Egypt. Food and Nutrition Sciences **7**: 472–484.

Dillard CJ, German JB, 2000. Review Phytochemicals: nutraceuticals and human health. Journal of Science and Food Agriculture **80**: 1744–1746.

El–Mohamedy RSR, Abdalla AM, 2014. Evaluation of Antifungal Activity of *Moringa oleifera* Extracts as Natural Fungicide against Some Plant Pathogenic Fungi In vitro. International Journal of Agricultural Technology **10**(4): 963–982

El-Sayed M, Mansour ME, Aly AE, Mohamed FE, Zannon AA, 1990. Effect of some disinfectants on larval haemolymph protein and free amino acids of the larvae of silkworm, *Bombyx mori* L. Egyptian Journal of Applied Science **5**(5):39–44.

Ezeamuzie IC, Ambakederemo AW, Shode FO, Ekwebelem SC, 1996. Antiinflammatory effects of *Moringa oleifera* root extract. International Journal of Pharmacognosy **34**(3): 207–212.

Fahey JW, 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional therapeutic and prophylactic properties. Part 1 Trees for Life Journal **1**: 5.

Fakurazi S, Sharifudin SA, Arulselvan P, 2012. *Moringa oleifera* hydroethanolic extracts effecively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules **17**: 8334–8350.

Gaceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F, 1992. Pharmacological properties of *Moringa oleifera* 2: screening for antispasmodic, anti-inflammatory and diuretic activity. Journal of Ethnopharmacology **36**(3): 233.

Goyal BR, Agrawal BB, Goyal RK, Mehta AA, 2007. Phytopharmacology of *Moringa oleifera* Lam.: an overview. Natural Product Radiance **6**(4): 347–353.

Haristory X, Fahey J, Scholtus I, Lozniewski A, 2005. Evaulation of the antimicrobial effects of several isothiocyanates on *Helicobacter pylori*. Planta Medica. **71**: 326–330.

Harvey WF, John UL, 1898. King's American Dispensatory. 19 Edition, Third Revision Vol.2, The Ohio Valley, Company.

Hsu R, Midcap S, Arbainsyah DWL, 2006. *Moringa oleifera*: Medicinal and Socio-Economical Uses. International Course on Economic Botany, National Herbarium Leiden, the Netherlands.

Ishaaya I, Moore I, Joseph D, 1971. Protease and amylase activity in larvae on the Egyptian cotton worm, *Spodoptera littoralis*. Journal of Insect Physiology **17**: 45–953.

Janick J, Paull RE, 2008. The encyclopedia

of fruit and nuts. CABI, Cambridge, UK, 509–510 pp.

Karagiorgou I, Grigorakis S, Lalas S, Makris DP, 2016. Polyphenolic burden and in vitro antioxidant properties of *Moringa oleifera* root extracts. Journal of Herbbed Pharmacology **5**(1): 33–38.

Karthikairaj K, Isaiarasu L, Sakthivel N, 2014. Efficacy of some herbal extracts on microbes causing flacherie disease in mulberry silkworm, *Bombyx mori* L. Journal of Biopesticides **7**: 89–93.

Kasolo JN, Bimenya GS, Ojok L, 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. Journal of Medicinal Plants Research **4**: 753–757.

Khattab MMA, Shakak MAS, 2012. Effect of frying on the physicochemical properties of *Moringa oleifera*, groundnut (Arachishypogaea) ail and their blends, Journal of Science and Technology **13**: 85–94.

Krishnaswami S, 1978. New technology of silkworm rearing. Central Sericulture Researches and Training, Institute Mysore Bulletin **2**:1–10.

Kumar R, Ramanathan G, Subhakaran M, Inbaneson S, 2009. Antimicrobial compounds from marine halophytes for silkworm disease treatment. International Journal of Medicine and Medical Sciences **1**(5): 184–191.

Mahesha HM, Rajashekhargouda R, Rayar SG, 1999. Effect of aqueous extracts of few botanicals with special reference to weeds on *Bombyx mori* L. The Proceeding of the XVIIIth International Sericultural Commission Congress, Cairo- Egypt, 12-16 October, 114–118 pp.

Mahmoud SM, 1988. Activation of silk secretion by silkworm, *Philosamia ricini* and *Bombyx. mori* after applying antibiotics. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Egypt.

Mbikay M, 2012. Therapeutic potential of *Moringa oleifera* leaves in chronichyperglycemia and dyslipidemia: a review. Front Pharmacology **3**:1–12.

Mesbah HA, El-Sayed Nagda AA, Yehia Wagiha H, Mahmoud Mona M, El-Karaksy IA, 2000. Effect of some natural plant products on the productivity of the mulberry silkworm, *Bombyx mori* L. Journal of the Advances in Agricultural Research **5**(2): 1331–1344.

Murugan K, Jeyabalan D, Senthikumar N, Senthilnathan S, Sivaprakasam N, 1998. Growth promoting effect of plant products on silkworm: A Biotechnological Approach. Journal of Scientific and Industrial Research **57**: 740–745.

Nigam SS, 1982. Antimicrobial activity of essential oils. Indian Perfumer **26**(2-4): 249–254.

Paliwal R, Sharma V, Sadhna S, 2011. Elucidation of free radical scavenging and antioxidant activity of aqueous and hydro-ehtanolic extracts of *Moringa oleifera* pods. Research Journal of Pharmacy Technology **4**: 566–571.

Parra JRP, 1991. Consumoe utilizaçào de alimentos por insetos. In: PANIZZI, A.R., Parra, J.R.P. Ecologia nutricional de Insetose suas Implicaçòes no manejo de pragas. 359 P. Manole, Sào Paulo, Brazil.

Patil RR, Chandrakala MV, Sukumar J, Channal HT, Kusugal S, Ankad G, 2005.

Utilization of Parthenium in silkworm rearing. Proceedings of II International conference on Parthenium management. University of Agricultural Sciences, Bangalore, India, 205–207 pp.

Prajapati ND, Purohit SS, Sharma AK, Kumar T, 2003. A Handbook of Medicinal Plants. Agrobios, India, pp. 554.

Price ML, 2000. The *Moringa* tree purification and activity assay of the coagulant protein from *Moringa oleifera* seed. Water Research **39**: 2338–2344.

Rajeswari K, Isaiarasu L, 2004. Influence of leaf, flower and pod extracts of *Moringa oleifera* on the growth and reproductive parameters of *Bombyx mori* L. Entomon **29**(4): 331–338.

Reitman S, Frankel S, 1957. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology **28**: 56–63.

Saeki K, Ozaki K, Kobayashi T, Ito S, 2007. Detergent alkaline proteases: enzymatic properties, genes, and crystal structures. Journal of Bioscience Bioengineering **103**(6): 501–508.

Sandhya B, Thomas S, Isabel W, Shenbagarathai R, 2006. Ethno medicinal plants used by the Valaian community of Piranmalai hille (Reserved Forest), Tamilnadu, India–A pilot study. African Journal of Traditional, Complementary and Alternative Medicines **3**(1): 101–114.

SAS Institute, 1988. SAS users guide. Statistics SAS Institute, Cary, N.C., USA.

Sharma V, Paliwal R, Janmeda P, Sharma S, 2012. Renoprotective effects of *Moringa oleifera* pods in 7, 12 dimethylbenz[a] anthracene exposed mice. Journal Chinese of Internal Medicine **10:** 1171–1178.

Siddhuraju P, Becker K, 2003. Antioxidants properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agriculture and Food Chemistry **51**: 2144–2145.

Suparna MK, Mallikarjun G, Ingalhalli SS, Shyamkumar V, Hooli AA, 2011. Role of antibacterial proteins in different silkworm strains against flacherie. The Bioscan **6**(3): 365–369.

Turk B, 2006. Targeting proteases: successes, failures and future prospects. Nature Reviews Drug Discovery **5**: 785–799.

Yousef Enas M, 2014. Evaluation of some compounds and plant extracts as disinfectants and nutritional additives and its effect on some biological, physiological aspects and silk production of mulberry silkworm, *Bombyx mori* L. Ph.D. Thesis, Faculty of Science, Zagazig University, Egypt.

Zannoon AA, Shadia MO, 1994. Efficiency of certain natural materials as mountages for mulberry silkworm, *Bombyx mori* L. Egyptian Journal of Applied Science **9**(8): 691–696.