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Effect of Dexatrol and Peppermint Oil as a Disinfectants on Some Biochemical Characters of Infected Silkworm *Bombyx mori*

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ABSTRACT

This study aimed to evaluate the effects of dexatrol antibiotic and peppermint oil with different concentrations as a disinfectants on infected silkworm *Bombyx mori* larvae with bacteria by studying their effects on some biochemical characters of larvae as the protein enzymes activity; aspartate transaminase (AST), alanine transaminase (ALT), Total Soluble Protein (TSP) and free radical biomarkers; lipid peroxidation (MDA) and Protein Carbonyl Content (PCC). It found that, most concentrations enhanced the enzymes activity and decreased the effects of free radical on lipid and protein of larval haemolymph compared with the control group, especially dexatrol concentration (0.4%) and peppermint oil concentrations (0.5 and 0.25%) revealed the best results significantly.

Key words: Silkworm, *Bombyx mori*, dexatrol, peppermint oil, biochemical characters

INTRODUCTION

Silkworm is prone to a number of diseases during its larval stage. The mortality rates become very high during infection spread. These diseases cause very serious problem that face silkworm rearing as they cause quantitative and qualitative reduction of natural silk crop production. Therefore, the produced cocoons by survivors have the depleted economic characters. Disease control can only be affected by prophylactic use of certain disinfectants or antibiotics (Saha *et al*., 1995; Sanakal *et al*., 1996; Deehu *et al*., 1997; Bali *et al*., 2008; Saad *et al*., 2012).

Antibiotics are widely used in sericulture industry as a component of bed disinfectants and as therapeutic applications against bacterial diseases, they are approved for four different purposes: Disease treatment, disease prevention, disease control and for health maintenance or growth promotion (Phillips *et al*., 2004; Subramanian *et al*., 2009).

The control of infectious diseases is seriously threatened by the continuous increase in the number of microorganisms that are resistant to the chemical antimicrobial drugs (Nenaah and Ahmed, 2011) and in view of high cost of chemicals and antibiotics and their hazardous consequences, plant extracts has been on the top priority for controlling diseases, recently there has been a concerted effort to promote the use of botanicals as possible alternatives to treat infectious diseases. These natural products were found to possess promising antimicrobial activities when applied alone or in combination with conventional antimicrobial drugs (Kumar *et al*., 1999; Jazani *et al*., 2007; Mohsenzadeh, 2007; Jazani *et al*., 2009; Chanda *et al*., 2011).

The components in essential oils vary not only with plant species but also in relation to climate, soil composition, part of the plant and age of the plant. Many essential oils are composed of a variety of terpenoid compounds. These substances are usually volatile and can be detected by the antennae or tarses of insects. The major terpenoids contained in essential oils are monoterpenoids (citronellal, linalol, menthol, pinene, mentona, carvona and limonene) (Paula *et al.*, 2004; Simoes and Spitzer, 2003).

Peppermint, is an aromatic and medicinal plant widely used in the food industry, perfumery and cosmetic, pharmacy and traditional medicine. Its essential oil displays antimicrobial activity against a range of bacteria and fungi. It has a high menthol content, contains also menthone and menthyl esters, particularly menthyl acetate and small amounts of many additional compounds including limonene, pulegone, caryophyllene and pinene (Bruneton, 1995).

Oxidant stress may lead to cellular injury and subsequent organ dysfunction (oxidative damage) by such mechanisms as oxidative damage to essential proteins, lipid peroxidation, DNA strand breakage (Darley-Usmar and Halliwell, 1996).

The process of uncontrolled lipid peroxidation in biological system may be associated with the loss of essential polyunsaturated fatty acids which represented the high content of cellular membranes constituents. This process leads to the formation of toxic hydroperoxide or other secondary products and causes extensive disturbance to the fine structure of biological membranes, thus affects the permeability and function of the membrane and extensive oxidation may lead to rupture of cellular membranes and concomitant release of destructive lysosomal enzymes (Abdel-Rahmaan, 1999).

Proteins constitute the major working force for all forms of biological work. Their exact conformation and pattern of folding are tightly connected to their activity and function. Reactive oxygen species are formed during normal metabolism and in higher fluxes under pathological conditions. They cause cellular damage, an important part of which is the oxidation of amino acid residues on proteins, forming protein carbonyls. The addition of carbonyl groups to amino acid residues in proteins is an oxidative modification of protein and involved in many physiological and pathological processes (Fagan *et al.*, 1999; Chevion *et al.*, 2000).

The present study aimed to evaluate the effects of dexatrol antibiotic and peppermint oil as a disinfectants on some biochemical aspects of the haemolymph of infected silkworm larvae *Bombyx mori*.

MATERIALS AND METHODS

The present study was carried out during the spring season of 2012 in the laboratory of Sericulture Research Department of Plant Protection Research Institute, Sharkia Branch, Agriculture Research Center.

Materials

- Mulberry silkworm, *B. mori* eggs (Egyptian hybrid Giza)
- Dexatrol antibiotic consists of dexamethasone (1 mg/1 mL), Neomycin {as sulphate} (3.5 mg/1 mL) and polymyxin-B-sulphate (6000 IU/1 mL) from Egyptian International Pharmaceutical Industries Company (E.I.P.I.Co.)

Four milliliter of antibiotic dissolved in 1 L distilled water to prepare a concentration of 0.4% and 3 mL of antibiotic to prepare a concentration of 0.3% and so the remaining concentrations to 0.1%.

- Peppermint oil (Cap. Pharm. Company) was used with 4 concentrations prepared by Harvey and John (1898)

Methods

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. Chicken egg cartons plates were used as montages for cocoon spinning (Zannoon and Shadia, 1994). Larvae under investigation were divided into two groups. Each group was divided into four subgroups. One group feed on mulberry leaves treated with dexatrol antibiotic concentrations and the other group feed on mulberry leaves supplemented with peppermint oil concentrations using 3 replicates (100 larvae) for each concentration. Mulberry leaves were dipped in the concentrations of both disinfectants for 5 min and left to dry then offered to larvae. Larvae were treated with disinfectants two times in both the 4th and 5th larval instars, all feeds/day at the first day and the middle of both instars. The control group of leaves was treated only with distilled water.

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), *Bombyx mori* artificially infected by spraying mulberry leaves with the concentration (15 ppm) of bacterial flacherrie (*Streptococcus pneumoniae*) and fed one time in the 2nd day of the 4th instar larvae.

Biochemical assays: Samples were made by removing one of the thoracic legs of the 5th instar 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 (Mahmoud, 1988). The tubes were kept at-20°C. The blood samples were centrifuged at 10000 rpm for 10 min at 5°C. The supernatant was immediately assayed to determine aspartate transaminase (AST), alanine transaminase (ALT) activities according to the method of Reitman and Frankel (1957), Total Soluble Protein (TSP) as described by Gornall *et al.* (1949), lipid peroxidation (MDA) according to the method of Sharma and Wadhwa (1983) and Protein Carbonyl Content (PCC) as described by Levine *et al.* (1990).

Statistical analysis: The obtained results were subjected to statistical analysis of variance and the data were presented as means according to Snedecor and Cochran (1982) methods using software COSTAT program.

RESULTS AND DISCUSSION

Alanine transaminase (ALT): Data exhibited a significant increase as shown in Fig. 1a of ALT means of larvae fed on mulberry leaves treated with dexatrol antibiotic and peppermint oil with different concentrations than control (0.90 µg Pyruvate/mL, SE = ±0.07) especially the 4th

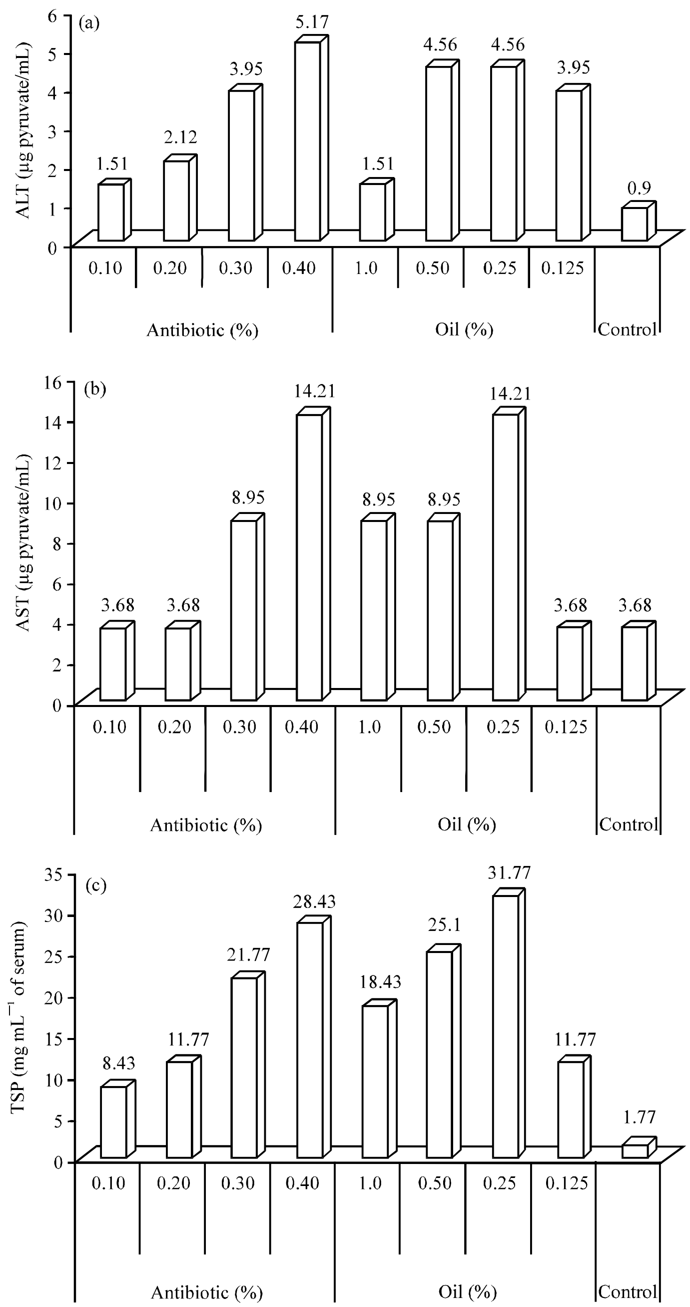

Fig. 1(a-c): Effect of dexatrol and peppermint oil on (a) Alanine transaminase (ALT), (b) Aspartate transaminase and (c) Total Soluble Proteins (TSP) of infected larval haemolymph

concentration (0.4%) of dexatrol (5.17 µg Pyruvate/mL, SE = ±0.15) and the 2nd and 3rd concentrations (0.5%, 0.25%) of peppermint oil (4.56 µg Pyruvate/mL, SE = ±0.34 and 4.56 µg Pyruvate/mL, SE = ±0.15) respectively, LSD 0.05 (conc.) = 0.863*** for each one.

Aspartate transaminase (AST): Obtained data in Fig. 1b, cleared that larvae fed on mulberry leaves treated with dexatrol antibiotic and peppermint oil with different concentrations recorded higher means of AST than control (3.68 μg Pyruvate/mL, SE = ±0.35) especially the 4th concentration (0.4%) of dexatrol (14.21 μg Pyruvate/mL, SE = ±0.48) and the 3rd concentration (0.25%) of oil (14.21 μg Pyruvate/mL, SE = ±0.51), LSD 0.05 (conc.) = 1.905*** for each treatment. According to the obtained results, using the disinfectants and antibiotic to the infected mulberry silkworm, *B. mori* increased the protein content as compared to the infected control group. These results may suggest that the disinfectants may stimulate the enzyme activity which influences the biochemical contents of the haemolymph of the silkworm, *B. mori*. The results are in partial accordance with El-Sayed *et al.* (1990) who stated that, disinfectants increased the amount of haemolymph protein of larval instars and in agreement with those of Ghada (2009, 2012) evaluated the effects of mulberry leaves supplemented with the botanical oils on silkworm *Bombyx mori*. The results proved that oils exhibited the highest activity of ALT and AST. Also, it was found that the activity of AST enzyme was higher than ALT enzyme.

Determination of Total Soluble Protein (TSP): As illustrated in Fig. 1c, larvae fed on mulberry leaves treated with the 4th concentration (0.4%) of dexatrol (28.43 mg mL^{-1} of serum, SE = ±0.68) and the 3rd concentration (0.25%) of peppermint oil (31.77 mg mL^{-1} of serum, SE = ±0.65) recorded the highest TSP values compared with the other concentrations and control (1.77 mg mL^{-1} of serum, SE = ±0.18), LSD 0.05 (conc.) = 2.656***.

The infection with bacterial diseases decreased the total protein content. These results explained with Azab (2003) found that, the infection with *B. thuringiensis* caused marked reduction in the total protein contents at different concentrations in the black cutworm. Ross *et al.* (1980) stated that, peppermint oil inhibited the growth *in vitro* of many kinds of bacteria such as *Staphylococcus aureus* and fungi. In parallel direction, Rietschel and Fowler (2001) reported that, neomycin is an antibacterial that is used widely in medical treatments. It is also commonly found in combination preparations with other antibacterials and corticosteroids, it has excellent activity against Gram-negative bacteria and has partial activity against Gram-positive bacteria.

Lipid peroxidation (MDA): The levels of lipid peroxidation of haemolymph of the infected larvae reared on mulberry leaves enriched with investigated antibiotic and oil with different concentrations compared with control were shown in Fig. 2a. A significant decrease were noticed in MDA contents associated with increased concentrations of dexatrol; the concentrations 0.3% (25.11 nol mL^{-1} of serum, SE = ±0.62) and 0.4% (20.78 nol mL^{-1} of serum, SE = ±0.41) compared with control (41.11 nol mL^{-1} of serum, SE = ±0.62), also, the concentrations of oil 0.5% (26.11 nol mL^{-1} of serum, SE = ±0.67) and 0.25% (23.89 nol mL^{-1} of serum, SE = ±0.52) revealed a significant decrease in MDA content compared with control, (LSD 0.05 (conc.) = 2.946***).

Niesink *et al.* (1995) reported that free radicals can induce a wide range of effects such as membrane damage, inactivation of enzymes and cell death. These results supported by the study of Andallu and Varadacharyulu (2003) observed that mulberry leaves significantly decreased lipid peroxidation and increased the activity of almost all the antioxidant enzymes in normal rats and Bouari *et al.* (2014) stated that peppermint could represent a potential source of natural antimicrobial products. Dexatrol combines two antibiotics, neomycin and polymyxin B which highly active against a wide range of Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *E. coli*, *Klebsiella* sp., *Neisseria* sp., *Proteus* sp., *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. It also contains dexamethasone, a potent and effective

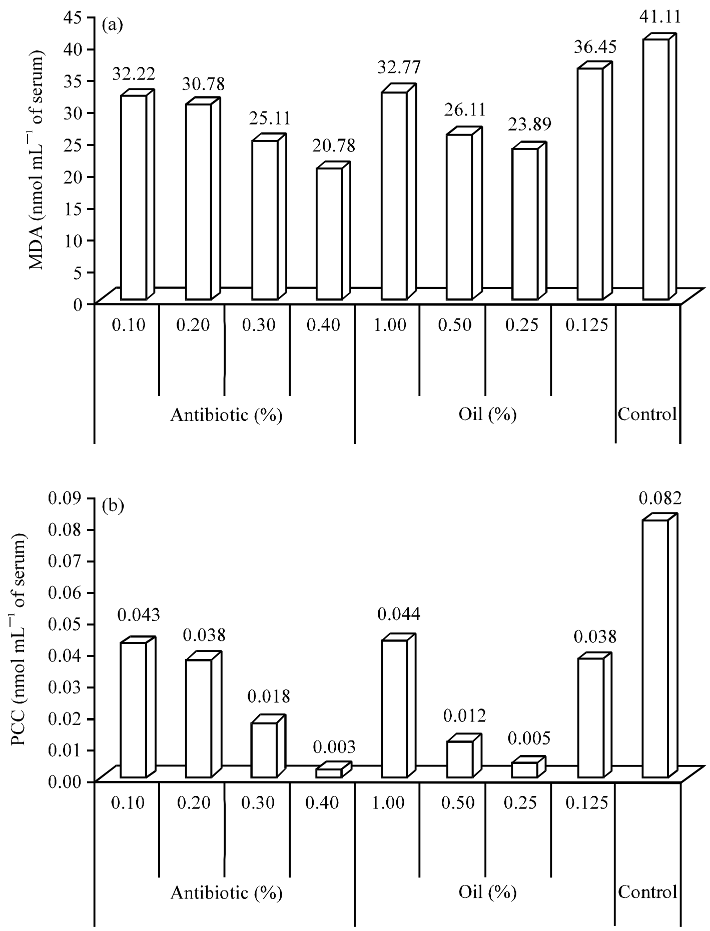

Fig. 2(a-b): Effect of dexatrol and peppermint oil on (a) Lipid peroxidation (MDA) and (b) Protein Carbonyl Content (PCC) of infected larval haemolymph

corticosteroid with anti-inflammatory action, thus controlling the undesirable phases of inflammation associated with bacterial infections (E.I.P.I.Co.).

Protein Carbonyl Content (PCC): As shown in Fig. 2b, all antibiotic and oil concentrations exhibited a significant decrease in the level of protein carbonyl content of infected larval haemolymph compared with control, especially larvae fed on mulberry leaves supplemented with the concentration 0.4% of dexatrol (0.003 nol mL^{-1} of serum, SE = ±0.0003) and the concentration 0.25% of peppermint oil (0.005 nol mL^{-1} of serum, SE= ±0.0003) which revealed significantly the least values compared with control group (0.082 nol mL^{-1} of serum, SE = ±0.004), LSD 0.05 (conc.) = 0.014***.

These results are in agreement with those of Sarker *et al.* (1995) who found that, the total protein content of silk gland increased by feeding larvae on mulberry leaves supplemented with different nutrients and Murugan *et al.* (1998) stated that, the extracts of *T. terrestris*, *B. diffusa* and *P. niruri* plants revealed more protein fractions and a higher level of proteins in the silk glands of silkworm. Alagumalai *et al.* (1991) found that, the antibiotics increased the larval weight and silk gland weights (Fig. 3).

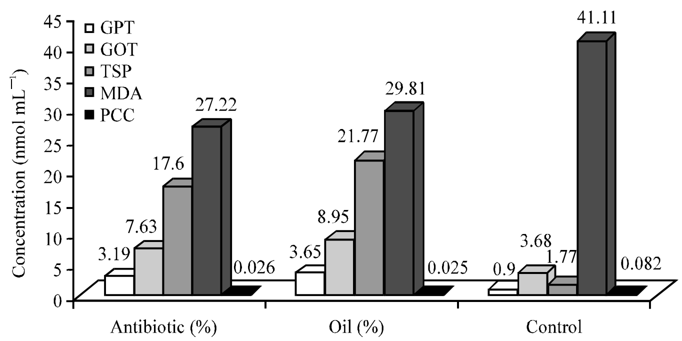

Fig. 3: A comparison among dexatrol antibiotic, peppermint oil as a disinfectants and control on biochemical aspects

CONCLUSION

Conclusively, the mechanism of increased silk production in silkworm larvae depending on amines transfer which involved in the uptake of used compounds from leaves by the body tissues and silk glands which resulted in the subsequent promotion of silk protein synthesis (Enas, 2014). So, the treated mulberry leaves with disinfectants, dexatrol antibiotic and peppermint oil have benefit effect on diseased silkworm larvae, especially with higher concentrations; increased the protein enzymes activity (ALT and AST) and Total Soluble Proteins (TSP) and decreased free radicals biomarkers of lipid and protein (MDA and PCC). So it could be recommended that, the use of the disinfectants, dexatrol antibiotic and peppermint oil inhibit the symptoms of bacterial diseases as compared to the infected control group.

REFERENCES

Abdel-Rahmaan, M., 1999. Physiological and biochemical effects of albendazole (*Antiparasitic drug*) on some experimental animals. M.Sc. Thesis, Zoology Department, Faculty of Science, Suez Canal University, Egypt.

Alagumalai, K. R. Ramaraj, M. Thiruvalluvan and N. Nagendran, 1991. Effect of antibiotics on larval, cocoon characters and fecundity of silkworm *Bombyx mori* L. Environ. Ecol., 9: 795-796.

Andallu, B. and N.C. Varadacharyulu, 2003. Antioxidant role of mulberry (*Morus indica* L. cv. Anantha) leaves in streptozotocin-diabetic rats. Clinica Chimica Acta, 338: 3-10.

Aneja, K.R., 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. 4th Edn., New Age International (P) Ltd., Chennai, India, ISBN-13: 9788122414943, Pages: 632.

Azab, A.M.H., 2003. Susceptability of different stages of the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) to the infection with the bacteria, *Bacillus thuringiensis*. Egypt. J. Applied Sci., 18: 382-390.

Bali, R.K., A. Koul and K. Ram, 2008. Evaluation of some low cost materials as silkworm bed disinfectants. J. Res. SKUAST-J., 7: 118-121.

Bouari, C., P. Bolfa, G. Borza, G. Nadas, C. Catoi and N. Fit, 2014. Antimicrobial activity of *Mentha piperita* and *Saturenja hortensis* in a murine model of cutaneous protothecosis. J. Med. Mycol., 24: 34-43.

Bruneton, J., 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisier Publishing, Paris, ISBN-13:9782743000288, Pages: 915.

Chanda, S., M. Kaneria and R. Nair, 2011. Antibacterial activity of *Psoralea corylifolia* L. seed and aerial parts with various extraction methods. Res. J. Microbiol., 60: 124-131.

Chevion, M., E. Berenshtein and E.R. Stadtman, 2000. Human studies related to protein oxidation: Protein carbonyl content as a marker of damage. Free Radic. Res., 33: S99-S108.

Darley-Usmar, V. and B. Halliwell, 1996. Blood radicals: Reactive nitrogen species, reactive oxygen species, transition metal ions and the vascular system. Pharmaceut. Res., 13: 649-662.

Deehu, P.S., R. Govindan, M.C. Deraiah and T.K.N. Swamy, 1997. Effect of antibiotics on growth and cocoon parameters of silkworm, *Bombyx mori* (L). L. Mysore J. Agric. Sci., 31: 41-46.

El-Sayed, M., M.E. Mansour, A.E. Aly, F.E. Mohamed and A.A. Zannon, 1990. Effect of some disinfectants on larval haemolymph protein and free amino acids of the larvae of silkworm *Bombyx mori* L. Egypt J. Applied Sci., 5: 39-44.

Enas, Y.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.

Fagan, J.M., B.G. Sleczka and I. Sohar, 1999. Quantitation of oxidative damage to tissue proteins. Int. J. Biochem. Cell. B., 31: 751-757.

Ghada, M.M., 2009. Studies on the role of some natural products on the biology, physiology and silk secretion on silkworm, *Bombyx mori* L. M.Sc. Thesis, Faculty of Agriculture Benha University, Egypt.

Ghada, M.M., 2012. Biological, physiological and technological studies on silkworm, *Bombyx mori* L. Ph.D. Thesis, Factuality of Agriculture Benha University, Egypt.

Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.

Harvey, W.F. and U.L. John, 1898. King's American Dispensatory. 19th Edn., Vol. 2, Ohio Valley Company, Virginia, USA.

Jazani, N.H., M. Zartoshti, S. Shahabi, Z. Yekta and S. Nateghi, 2007. Evaluation of the synergetic effect of water soluble extracts of green tea (*Camellia sinensis*) on the activity of ciprofloxacin in urinary isolated *E. coli*. J. Biol. Sci., 7: 1500-1503.

Jazani, N.H., M. Zartoshti, H. Babazadeh, N. Ali-Daiee, S. Zarrin and S. Hosseini, 2009. Antibacterial effects of Iranian fennel essential oil on isolates of *Acinetobacter baumannii*. Pak. J. Biol. Sci., 12: 738-741.

Krishnaswami, S., 1978. New technology of silkworm rearing. Cent. Sericulture Res. Training Inst. Mysore Bull., 2: 1-10.

Kumar, S., J. Singh and A. Sharma, 1999. Asian region inventory of medicinal and aromatic plants and polyherbal formulations. Department of Biotechnology, New Delhi, India, pp: 191.

Levine, R.L., D. Garland, C.N. Oliver, A. Amici and I. Climent *et al.*, 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol., 186: 464-478.

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

Mohsenzadeh, M., 2007. Evaluation of antibacterial activity of selected Iranian essential oils against *Staphylococcus aureus* and *Escherichia coli* in nutrient broth medium. Pak. J. Biol. Sci., 10: 3693-3697.

Murugan, K., D. Jeyabalan, N.S. Kumar, S.S. Nathan and N. Sivaprakasan, 1998. Growth promoting effects of plant products on silk worm. J. Sci. Ind. Res., 57: 740-745.

Nenaah, E.G. and M.E. Ahmed, 2011. Antimicrobial activity of extracts and latex of *Calotropis procera* (Ait.) and synergistic effect with reference antimicrobials. Res. J. Med. Plant, 5: 706-716.

Niesink, K.J., J. De Vries and M.A. Hollinger, 1995. Toxicology: Principles and Applications. 1st Edn., Published by CRC Press, Netherlands, ISBN: 13-9780849392320, Pages: 1312.

Paula, J.P., P.V. Farago, L.E.M. Checchia, K.M. Hirose and J.L.C. Ribas, 2004. Atividade repelente do Oleo essencial de *Ocimum selloi* Benth. (variedade eugenol) contra o *Anopheles braziliensis* chagas [Repellent activity of *Ocimum selloi* Benth. essential oil (variety eugenol) against *Anopheles braziliensis* chagas]. Acta Farmaceutica Bonaerense, 23: 376-378, (In Portuguese).

Phillips, I., M. Casewell, T. Cox, B. Groot and C. Friis *et al*., 2004. The use of antibiotics in food animals pose a risk to human health. J. Antimicrob. Chemother., 54: 276-278.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Rietschel, R.T. and J.F. Fowler, 2001. Fisher's Contact Dermatitis. 5th Edn., Lippincott Williams and Wilkins, Hagerstown, Maryland, Pages: 862.

Ross, S.A., N.E. El-Keltawi and S.E. Megalla, 1980. Antimicrobial activity of some Egyptian aromatic plants. Fitoterapia, 51: 201-205.

Saad, M.I.S., H.M. Eman and A.A. Zannoon, 2012. Effect of some antibiotics on the biological and silk production of mulberry silkworm, *Bombyx mori* L. Egypt. J. Agric. Res., 90: 537-545.

Saha, A.K., M.S. Rahaman, B.N. Saha and D.K. Das, 1995. Effect of antibiotics on the growth and rearing performance of silkworm, *Bombyx mori*. J. Asiatic Soc. Bangladesh Sci., 21: 289-292.

Sanakal, R.D., S.S. Ingalhalli, K.K. Singh, S. Basavarajappa, S.B. Hinchigeri and C.J. Savanurmath, 1996. Infectious Flacherie of the silkworm *Bombyx mori* in Northern Districts of Karnataka, India. Indian J. Sericulture, 35: 90-94.

Sarker, A., M. Haque, M. Rab and N. Absar, 1995. Effect of feeding mulberry (*Morus* sp.) leaves supplemented with different nutrients to silkworm (*Bombyx mori* L.). Curr. Sci., 2: 185-188.

Sharma, S.P. and R. Wadhwa, 1983. Effect of butylated hydroxytoluene on the life span of *Drosophila bipectinata*. Mech. Ageing Dev., 23: 67-74.

Simoes, C.M.O. and V. Spitzer, 2003. Oleos Volateis. In: Farmacognosia: Da Planta ao Medicamento, Simoes, C.M.O., E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz and P.R. Petrovick (Eds.). 5th Edn., UFRGS., Porto Alegre, pp: 467-496.

Snedecor, G.W. and W.G. Cochran, 1982. Statistical Methods. Iowa State University Press, USA.

Subramanian, S., P. Mohanraj and M. Muthuswamy, 2009. Newparadigm in silkworm disease management using probiotic application of *Streptomyces noursei*. Karnataka J. Agric. Sci., 22: 499-501.

Suparna, M.K., G. Mallikarjun, S.S. Ingalhalli, V.S. Kumar and A.A. Hooli, 2011. Role of antibacterial proteins in different silkworm strains against flacherie. The Bioscan, 6: 365-369.

Zannoon, A.A. and M.O. Shadia, 1994. Efficiency of certain natural materials as mountages for mulberry silkworm, *Bombyx mori* L. Egypt. J. Applied Sci., 9: 691-696.