The Effect of Homeopathic Drug and Essential Oil against Greater Wax Moth, *Galleria mellonella* L.

A.H. Almadani, C.J. Hiware

**ABSTRACT**

The honeybee is known since ancient time as an important factor for increasing the yield of various crops and produces different products such as propels pollen, royal jelly, bee wax and honey. The honeybees get infected by some pests such as the lesser wax moth which is a highly destructive insect that attacks and destroy bee wax combs especially those in storage and can cause substantial losses to combs. The greater wax moth (GWM) *Galleria mellonella* L. is one of the most devastating pest. The influence of Homeopathic drug Iodium and Essential Oil as Thyme on the biology of the greater wax moth was studied in the laboratory. The result shows that, with an increase in the concentrations and relative periods there was a corresponding increase in the total mortality of greater wax moth larvae. However, the hatching decreased with increase in the concentration of Homeopathic drug Iodium and Essential Oil Thyme when compared to control significantly.

**Key words:** Essential Oil, *G. mellonella*, Homeopathic drug.

**INTRODUCTION**

Nowadays farmers are taking keen interest in apiculture in which they get the additional income with good productivity of crops. Honey bees are affected by different sorts of enemies. The greater wax moth *G. mellonella* L. is one of the most devastating insects. Which causes significant damage to apiaries to bee hives resulting, that lead to financial losses for beekeepers (Kapil and Sihag, 1983 and Hanumanthaswamy *et al*., 2013). The total honey production in India is estimated to be 65,000MT during 2010-2011 (National Honey Board, 2011 and Krishnasree and Mary Ukkuru P. 2015).

The honey bee enemies create serious problems, which must be met not only by the bee keeper but also by the bees themselves. The occurrence of such biological constraints are many and at great variance across locations and countries. In most instances, the appearance of enemy is abrupt and instantaneous which plays havoc to honey bee colonies in the apiaries. Honey bees are affected by several natural enemies, Among them, the grater wax moth (GWM) is the most dangerous pest of the honey bee, *Apis mellifera* L. The wax moth feeds on pollen stored in combs as well as they feed on wax of bee colonies (Gulati and Kaushik, 2004, Milan, 1970 and Mohamed *et al*., 2014). There are two types of moth, Greater wax moth, *Galleria mellonella* L. and lesser wax moth, *Acheroria gresilla* L. (Owayss and Abd-Elgayed, 2007). Bee wax of all the species of *Apis* in India is attacked by wax moths (Nagaraja and Rajagopala, 2009 and Hanumanthaswamy *et al*., 2013). Wax moth is found in all honey bee breeding places however they are more active in the warmer and at temperatures near about 27°C (Charrière and Imadorf, 1997 and Al-Omairy, 2013).

In India infestation observed throughout the years in both higher (Sharma *et al*., 2013) and lower altitudes, however, the highest infestations are observed between May to September month. (Hanumanthaswamy *et al*., 2013).

There are many possibilities and manipulations used for controlling wax moth including in the hive and others in stored wax as physical, chemical methods and technical, biological processes. Sulphur fumigation, acetic acids and formic acids evaporation and applying paradichlorobenzene are practiced widely (Calderone, 2000 and Owayss and Abd-Elgayed, 2007).

Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment (Arnason *et al*., 1989; Kwon *et al*., 1996; Ahn *et al*., 1977 and Koul *et al*., 2008 and Mohamed *et al*., 2014). Natural products of plants and plant derivatives are an alternative agent to currently use for insect control because they constitute rich sources of bioactive chemicals. They are often active against insect species. They are biodegradable to non-toxic products. Additionally, plant-derived materials are found to be highly effective against insecticide resistant insect pests (Arnason *et al*., 1989; Kwon *et al*., 1996; Ahn *et al*., 1997 and Koul *et al*., 2008 and Mohamed *et al*., 2014). So that many investigators initiated large screening efforts poisonous effects to use them as
insecticides (Khambay et al., 2002; El-Shazly and Hussien, 2004; Prowse et al., 2006; Malarvannan and Subashini, 2007 and Khalaf et al., 2009 and Mohamed et al., 2014).

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Wink, 1993 and Kim et al., 2003). Several wild plant extractsisolated active compounds have been shown to act as potent acute or chronic insecticides (Emara et al., 2002; Tripathy and Singh, 2005; Moawad and Ebadah 2007 and Mohamed et al., 2014). Some facts show that the use of synthetic chemicals to control insects several obvious concerns related to environment and human health. So, there is a growing demand for alternative repellents or natural products. These products possess good efficacy and are environmentally friendly. Essential volatile oils from plants belonging to several species have been extensively tested to assess their repellent properties as a valuable natural resource (Grad, 2010; Nerio et al., 2010 and Mohamed et al., 2014). Essential volatile oils are complex mixtures isolated from aromatic plants which may possess antioxidant and anti-inflammatory activities of interest in their food and cosmetic industries as well as in the health field (Miguel, 2010 and Mohamed et al., 2014). In Nature, essential oils play an important role in the attraction of insects to promote the dispersion of pollens and seeds or to repel other ones. In addition, essential oils may also act as anti-bacterial, antiviral, antifungal, insecticides, herbicides, or have feeding deterrent effects against herbivores by reducing their appetite for such plants. Essential oils have also an important role in allopathic communication between plants (Ibrahim et al., 2001 and Bakkali et al., 2008 and Mohamed et al., 2014).

In recent years, homeopathic remedies are prescribed by more than 400,000 health care professionals worldwide, including paediatricians, family physicians, plastic surgeons, dentists and orthopaedic doctors. Homeopathic remedies are derived from natural substances that come from plants, minerals, or animals. Homeopathy works with your whole body to ease symptoms and make you well. Traditional medicine, on the other hand, simply targets and opposes the symptoms. Homeopathy uses just five ingredients in the production process: water, alcohol, sucrose, lactose and the natural medicine additionally, homeopathic medicines are simple to administer, even to children. Natural ingredients are used to produce homeopathic medicine. Unlike conventional drugs the remedies contain no toxic chemicals, making them safe for both, body and the environment.

Recently, research on homeopathic drugs has increased attention from both industrial and academic circles due to a growing interest in green consumerism. In present study we have made the attempt to use homeopathic drug Laschesis30 against fifth in star larvae and eggs hatching of (GWM), Galleria mellonell L. which seem to be safer and less contaminant to bees and humans. Also, the drug is cheap, easily available in the medical shop and easy to use.

**MATERIALS AND METHODS**

**Insect rearing technique**

Wax moth infected comb were collected from weak hives of apriaries around Aurangabad. These were kept in large boxes with a dimension of 50 x 30 x 30 cm size till becomes having a lot of larvae at 5th instar, as well as the cocoons has taken near about two weeks till emergence as an insect. In addition the adult has taken more than 8 days for female and more than 25 days for female till death. The laboratory strain larvae of (Galleria mellonella L.) were reared on a natural culture composed of honey wax content a little honey and pollen and reared at room air temperature 25-30° C and 60 ±5% R.H., the current experiment started with the 5th instar larvae, these larvae are transferred to vials (333ml size, covered with a tight, non-perforated cover) to start expose on the aroma of treatments inside it, i.e. Lechesis200 put in a small rectangular pieces paper to study the mortality.

**Experimental technique**

**Homeopathic drugs**

One commercially available Homeopathic drug Lechesis200is tested in this study. The Homeopathic drug was purchased from SBL PVT. Ltd. procured from local Central Homeopathic Pharmacy Shop, Dalal wadi, Aurangabad, M.S. India.

**Volatile oils**

Thyme volatile oils (thymus) were tested in this study. The tested oils were purchased as pure oil (Branched in India) from Pharaonic Company of medicinal and aromatic oils (R, K’s AROMA SHOP). Unit 4/20 Ajay Ind. Estate, Anjirwadi B, Mazgaon, Mumbai. The oils were extracted from the dried plants of Thyme by steam distillation.

**LC₉₀ tests**

Whatman filter paperswere cut into pieces (3x10 cm) and different treatments with (0.3, 0.6, 1.2, 2.4 ml/Litre air) Homeopathic drug Iodium200 and essential oil Thyme concentrations were applied on the Whatman filter papers, The toxicity of four different concentrations of the Laschesis 30 has been tested in vials, each of them containing 10 of larvae, the four tested concentrations were prepared by putting 0.1ml of drug on the paper so dangle it in the vial (size 333 of air), so it means it has given (0.3ml/ Litre air) of the first concentration, as well as we put 0.2 ml of drug in the same size of vial to give us (0.6ml/ Litre air) of the second concentration, in addition we put 0.4 ml of drug in the same size of vial to give us (1.2ml/ Litre air) of the third concentration, in final one we put 0.8 ml of drug in the same size of vial to give us (2.4ml/ Litre air) of the fourth concentration. Treated papers were dangle inside the treatments vials, after 24 hours from the exposure period, numbers of dead and alive larvae were counted, the 10%, 50% and 90% lethal concentrations (LC₁₀, LC₅₀ and LC₉₀) values were assessed by Probit analysis (Finney, 1971).
The Effect of Homeopathic Drug and Essential Oil against Greater Wax Moth, *Galleria mellonella* L.

### Mortality study

To study the mortality of all treatments in this study (0.3, 0.6, 1.2, 2.4 ml/Litter air) concentrations of Homeopathic Iodium200 and essential oil Thyme were used, just added control group of larvae, the control group contains non-treated papers and distributed randomly. Three replication were used per treatment, in each replication 10 larvae of 5th instar were taken. All larvae in this study were breathing the aroma that came from treated dangling papers; they never touch or eat the treatments. Then after the specified times for each treatment the papers were removed out from the vials and the observation are made for all treatments, after 1, 2, 7 and 14 days the larval mortalities were counted and recorded.

### Hatchability study

To study the effect of Laschesis200 on egg hatchability we took five males and five females in each vial, it used tens of vials to collect the enough eggs for hatching, then let it meet within one day so they started lay eggs mostly at the second day, daily collected eggs from all the frequents vial then put it at the air condition (at 28-30°C and 60 ±5% R.H.) till the 8th day when the larvae became clear inside the egg and started move inside it (whereas the hatched eggs be at 10th day approximately), then checked all eggs by microscope under 10x, then selected all valid eggs and moved to treatment vials (100 eggs/vial), so Lechesis200 put by the same previous concentrations (0.3, 0.6, 1.2, 2.4 ml/Litter air) on the paper and dangle it inside the vial to let the larvae inside the eggs breath the aroma not touch the eggs and one control group of eggs was put for comparison. The control group contains non-treated papers and distributed randomly also, three replicates were used as per the treatment, in each replicate 100 eggs were used. For egg hatching average period repaired was less than three days, on third day the hatched and unhatched eggs were counted and hatching percent was calculated. Data were analysed by using the Analysis of Variance (ANOVA) technique and the means were separated using Duncan multiple range tests (P> 0.05) (Steel and Torrie, 1980).

### RESULTS AND DISCUSSION

The effects of homeopathic and essential oil against GWM

The results of LC50 values of homeopathic (Iodium200) and essential oil (Thyme) are shown in (Table 1 and Fig 1). The LC50 of Iodium200 and Thyme was applied to the 5th larval instar of GWM and at hatched stage after exposure period 16 hours for this drug. Whereas after 24 Hours, survived larvae were monitored for latent effects on lethal concentration and counted the hatched eggs. Comparison of the estimated LC50 values of homeopathic and essential oil for larva recorded at (Table1) indicated that Iodium200 homeopathic is significantly more toxic to *G. mellonella* (0.67 ml/litre) than Thyme which was reported in this study (4.5 ml/litre).
The Effect of Homeopathic Drug and Essential Oil against Greater Wax Moth, *Galleria mellonella* L.

**Table 2:** Effect of Iodium200 and Thyme on Mortality and hatchability Wax Moth.

| Concentrations (ml/liter air) | 24hrs Hatching (%) | 24hrs Larval mortality (%) | 48hrs Larval mortality (%) | 1week Larval mortality (%) | 2weeks Larval mortality (%) |
|-------------------------------|--------------------|-----------------------------|---------------------------|--------------------------|----------------------------|
| Control                       | 99±0.577           | 0.00±0.00                   | 0.00±0.00                 | 0.00±0.00                | 3.3±3.33                   |
| Iodium200                     | 3.58±0.783         | 44.3±13.54                  | 45.1±13.32                | 45.5±13.20               | 49.8±12.10                 |
| Thyme                         | 0.75±0.411         | 1.7±1.12                    | 25±5.15                   | 45±6.80                  | 55±7.74                    |

**Mortality of 5th instar larvae and eggs hatched**

The percentages observed mortality were significantly deferment between Homeopathic of Iodium200 and Essential oil of Thyme. Whereas the percentage of larval mortality of GWM was (49.8%) when using Iodium200 compare than Thyme (55%) and untreated control (3.3%) after two weeks (Table 2).

In this present study is seen that the GWM was greatly influenced by the homeopathic drugs and essential oils, whereas the hatching effected by using Iodium200 and Thyme, the result showed the hatching was significantly decreased (8, 3, 3, 1%), (3, 2, 1, 1%) with increasing the concentration (0.3, 0.6, 1.2, 2.4 ml/litre air) once using Iodium200 and Thyme respectively and compare than untreated control that showed the highest hatched (98%) (Fig 2).

Results of this research showed that percentage of larval mortality was increased with the measurement time that is taken and the concentration for Homeopathic and Essential oils. Homeopathic and Essential oils was increased with the measurement time that is taken. Whereas Iodium200 and Thyme caused (44.3 and 1.7%) mortality in insect population after 24 h compared to 49.8 and 55 % mortality after 2 weeks respectively and untreated control 3.3% (Table 2).

Homeopathic of Iodium200 and Essential oil of Thyme at lowest concentration (0.3 ml/litre air) caused (0.3, 3.3 %) mortality in GWM population after 48h and compared to (100,30%) Cumulative larval mortality at the highest concentration (2.4 ml/litre air) respectively and 3.3% for untreated control (Table 3). Whereas, the Cumulative larval mortality was increased depends on the time of measurements has taken, compare to the measurement has taken after one day (Table 3).

Comparing the LC$_{50}$ values of present investigation with LC$_{50}$ values of mentioned essential oil indicated that essential oils of Thyme has toxic to *G. mellonella*. Results of this research clearly illustrated that insects varied in their susceptibility to various essential oils that probably referring to insecticidal ability of their active constituents. These results agreement with Pavela (2005) that tested thirty-four essential oils for insecticidal activity against larvae *Spodoptera littoralis*. Thyme essential oils was toxic to the third instar of *S. littoralis larvae*.

Many homeopathic drugs and their components were registered increasing on mortalities in silk worm when they used Iodium200 in their food (Avhad and Hiware, 2015).
Recent studies to control severe bee disease e.g., foulbroods using natural products are highly considered (Williams et al. 1998). From the obtained results it is clear that the Lachesis200 showed obvious effects on GWM compared to untreated control. In general, Iodium200 showed marked effects on these biological aspects. In conclusion, we recommend the use of Iodium200 Homeopathic drug with (2.4 ml/litre air) to protect stored wax combs from infestation with wax moths.

Employing the synthetic insecticides remains undesirable effects as mammalian toxicity, food chain disruption and increases resistance in pests (Regnault, 1997). Natural compounds could be efficient alternatives for conventional fumigants because of their low toxicity to mammals, fast degradability properties and regional availability (Hosseinipour et al., 2009; Abbasipour et al., 2009 and Mahmoudv and et al., 2011). Finally, in this work, we attempted to control GWM with Iodium200 homeopathic which seem to be safer and less contaminant to bees and humans. Also, these materials are cheap, available to beekeepers and could be used to control other hive infestations.

ACKNOWLEDGEMENT

The authors are thankful to all the beekeepers from Aurangabad district and to the University authorities, Head, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University Aurangabad, India, for providing necessary laboratory facilities during this work.

REFERENCES

Abbasipour, H., Mahmoudvand, M., Deylami, A. and Hosseinipour, M.H. (2009). Fumigant toxicity of essential oils of Rosemarinus officinalis L. and Eucalyptus camaldulensis Deh. against some stored products pests, proceeding of the 6th Asia-Pacific Congress of Entomology, Entomology in Health, Agriculture and Environment, Beijing, China.

Ahn, Y.J., Kwon, M., Park, H.M. and Han, C.G. (1977). The potent insecticidal activity of Ginkgo biloba-derived triactanon- etepernes against Nilaparvata lugens. In phytochemical pest control Agents; Hedin, [P. Hollingsworth, R., Miyamoto, J., Masler, E., Thompson., (Eds)]; ACS Symposium series 658; American Chemical Society: Washington, DC., pp. 90-105.

Al-Omairy, K.O. (2013). Effect of Some Plant Species Volatile Oils in Some of the Qualities Life of Greater Wax Warm Galleria Mellonella L. (Lepidoptera: Pyralidae). Iraqi Journal of Agricultural Sciences. 44(3): 367-372.

Aranson, J.T., Philogne, B.J.R. and Morand, P. (1989). Insecticides of plant origin; ACS symposium series. 387; Amer. Chem. Soc., Washington, DC, pp.164-172.

Avhad, S.B. and Hiware C.J. (2015). Impact Assessment of Homeopathic Drugs Iodium200 and Lachesis on Larvae, Cocoon and Post-Cocoon Characteristic of Bombyx mori L. International Journal of Research Studies in Biosciences (IJRSB). 3(4): 93-97.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M.M. (2008), Biological effects of essential oils- a review. Food Chem. Toxicol. 46: 446-475.

Calderone, N. (2000). IPM- wax moths, mice, wasps and robber bees. (Bee Culture Magazine. Jan. Issue).

Charrière, J. and Imadorf. A. (1997). Protection of honeycombs from moth damage. Swiss Bee Research Center, Federal Dairy Research Station. Liefeld, CH- 3003 Bern. Communication No. 24.

El-Shazy, A.M. and Hussein, K.T. (2004). Chemical analysis and biological activities of the essential oil of Teucrium leucocladum Boiss. (Lamiaceae). Biochem. Syst. and Ecology. 32(7): 665-674.

Emara, S., Bakr F.R., EL-Bermawy S., Abulyazid I. and Abdelwahab, H., (2002). Biological effects of four botanical extracts on the different developmental stages of cotton leaf worm. Spodoptera littoralis. 2nd Int. Conf. Plant Protection Research Institute, Cairo, 1: 904-916.

Finney, D.J. (1971). Probit analysis. (Cambridge Univ. Press, London, 3rd ed. 318 pp).

Grad, A.A. (2010). Biological properties of essential oils. An update. Magistra der Pharmazie (Mag.pharm.) Univ. Wien. 61 pp.

Gulati, R. and Kaushik, H.D. (2004), Enemies of honey bees and their management - a review, ARCC4253, Agric. Rev. 25 (3): 189-200.

Hanumanthaswamy, B.C., D. Rajagopal and Basavaraju, B.S. (2013). Influence of temperature and relative humidity on the development of greater wax moth Galleria mellonella L. (Pyralidae: Lepidoptera). Current Biotic. 7(3): 202-208.

Hosseinipour, M. H., Askarianzadeh, A., Moharramipour, S. and...
The Effect of Homeopathic Drug and Essential Oil against Greater Wax Moth, *Galleria mellonella* L.

Jalali, J. (2009). Fumigant toxicity of essential oils of *Artemisia unuua L.* (Asteraceae) against two stored product coleopteran insect pests. Proceeding of the 6th Asia-Pacific Congress of Entomology, Entomology in Health, Agriculture and Environment, Beijing, China.

Ibrahim, M.A., Kainulainen, P., Aflateni, A., Tiilikala, K., Holopainen, J.K. (2001). Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. Agr. Food Sci. Finland. 10: 243-259.

Kapil, R.P. and Sihag, R.C. (1983). Wax Moth and it’s control. Indian Bee J. 45(2/3): 47-49.

Khalaf, A.A., Hussein, K.T. and Shoukry, K.K. (2009). Biocidal Activity of two botanical volatile oils against the larvae of *Synthesomyia nudiseta* (Wulp) (Diptera: Muscidae). Egypt Acad. J. biology. Sci. 2(1): 89-101.

Kambay, B.P.S., Beddie, D.G. and Simmonds, M.S.J. (2002). An insecticidal mixture of tetramethyl cyclohexanedi one isomers from Kunzea ambigua and Kunzea bacteria. Phytochemistry. 59(1): 69-71.

Kim, S.I., Roh, J.Y., Kim, D.H., Lee, H.S. and Ahn, Y.J. (2003). Insecticidal activities of aromatic plant extracts and essential oils against *Staphylius oryzae* and *Callosobruchus chinensis*. Journal of Stored Products Research. 39: 293-303.

Koul, O., Walia, S. and Dhalwal, G.S. (2008). Essential Oils as Green Pesticides: Potential and Constraints. Biopestic. Int. 4(1): 63-84.

Krisnasree, V. and Mary Ukkuru, P. (2015). Quality evaluation of Indian bee (Apis cerana indica F.) honey in perception to enhance market potentiality. Asian J. Dairy and Food Res. 34(3): 243-246.

Kwon, M., Ahn, Y.J., Yoo, J.K. and Choi, B.R. (1996). The potent insecticidal activity of extracts from *Ginkgo biloba* leaves against *Nilaparvata lugens* (Homoptera: Delphacidae). Appl. Entomol. Zool. 31(1): 162-166.

Mahmoudvand, M., Abdasipour, H.H., Hosseinpour, M.H., Rastegar, F. and Basij, M. (2011). Using Some Plant Essential Oils as Natural Fumigants against Adults of *Callosobruchus Maculatus* (F.) (Coleoptera: Bruchidae). Mun. ENT. Zool. 6: 150-154.

Malarvannan, S. and Subashini, H.D. (2007). Effect of *Dodonaea Angustifolia* crude extract on biochemical profile of *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). Biochem. and Cellular Archives. 7 (1): 1-8.

Miguel, M.G. (2010). Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review. Molecules. 15: 9252-9287; doi: 10.3390/molecules15129252.

Milan, V.G. (1970). Moth pests of honey bee combs. Glean. Bee Culture, 68: 424-428.

Moawad, S.S. and Ebadah I. M.A. (2007). Impact of some natural plant oils on some biological aspects of potato tuber moth *Phthorimaea operculella*, (Zeller) (Lepidoptera: Gelechiidae). Rese. J. Agric. and Biol. Sci. 3(2): 119-123.

Mohamed, H.F., El-Naggar, S.E., Elbarky, N.M., Ibrahim, A.A. and Salama, M.S. (2014). The impact of each of the essential oils of marjoram and lemon. IOSR Journal of Pharmacy and Biological Sciences. 9(5): 92-106.

Nagaraja, N. and Rajagopal, D. (2009). Honey Bees: diseases, parasites, pests, predators and their management. MJ Publishers, Chennai, India.

Nerio, L.S., Olivero-Verbel, J. and Stashenko, E. (2010). Bioreosour Technol. 101: 372.

Owayys, A.A. and Abd-Elgayed, A.A. (2007). Potential Efficacy of Certain Plant Volatile Oils and Chemicals against Greater Wax Moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). Bull. ENT. Soc. Egypt. Econ. Ser. 33: 67-75.

Pavela, R. (2005). Insecticidal activity of some essential oils against larvae of Spodoptera littoralis. Fitosanitaria. 76(7/8): 691-696.

Prowse, G.M., Galloway, T.S. and Foggco, A. (2006). Insecticidal activity of garlic juice in two dipteran pests. Agr. Entomol. 8(1): 1-6.

Regnault R.C. (1997). The potential of botanical essential oils for insect pest control. Integrated Pest Management Reviews. 2: 25-34.

Sharma, N., Vashishth, S. and Sharma, P.K. (2013). Diversity and distribution of pests and predators of honey bees in Himachal Pradesh, India. Indian J. Agric. Res. 47 (5): 392-401.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and Procedures of Statistics. 2nd Ed. New York: McGraw-Hill.

Tripathy, M.K. and Singh, H.N. (2005). Synergistic effect of certain vegetable oils to the efficacy of synthetic pyrethroids for the control of *Helicoverpa armigera* (Hubner). Agric.Sci. Digest. 25(1): 1-5.

Williams, J. R., Peng, C.Y.S, Chuang, R.Y., Doi, R.H. and Mussen, E.C. (1998). The inhibitory effect of Azadirachtin on Bacillus subtilis, Escherichia coli and Paenibacillus larvae, the causative agent of American Foulbrood in honeybee, *Apis mellifera* L. J. Inverteb. Pathol. 72: 252-257.

Wink, M. (1993). Production and application of phytochemicals from an agricultural perspective. In: Phytochemistry and Agriculture, [Van Beek, T.A., Breteler, H. (Eds.)], 34: 171-213.