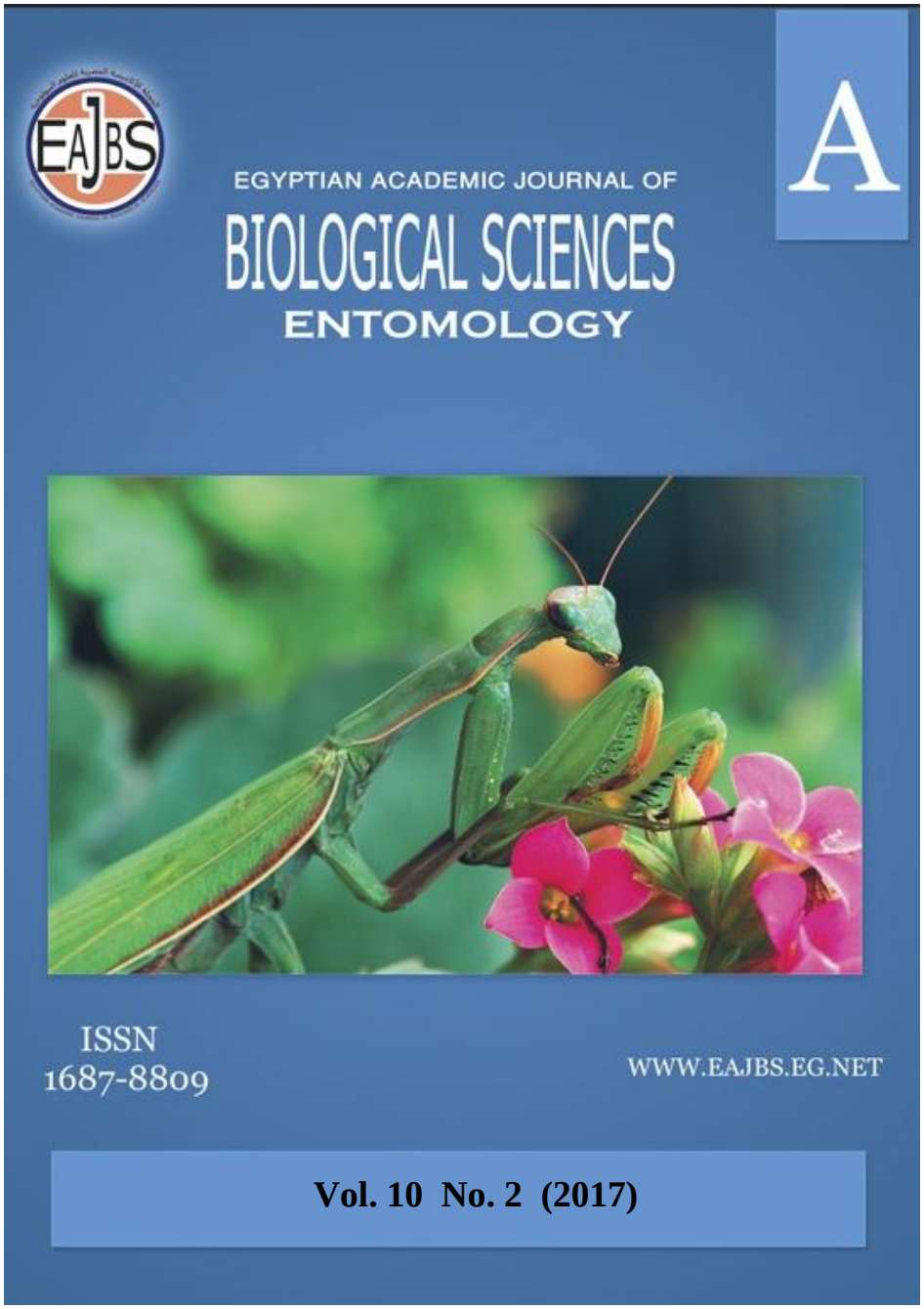

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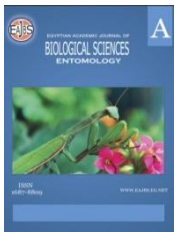

Role of Some Wild Herbal Extracts in Management of American Foulbrood in Honeybee Colonies

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ABSTRACT

American foulbrood (AFB) is one of the most contagious and dangerous among honey bee diseases. The causative agent is the Gram-positive bacterium, *Paenibacillus larvae.* The crude methanolic extracts of three wild herbs and tylosin antibiotic were tested against artificially AFB-infected honey bee colonies. The laboratory tests revealed the safety of these crude herbal extracts for adult honey bee workers. *Malcolmia pygmaea* (Cruciferae) extract was the most potent in control the AFB disease followed by *Marrubium alysson* (Labiatae) and *Lobularia arabica* (Cruciferae) was the least effective herb. Tylosin antibiotic was the weakest against the disease control, indicating to the bacterial resistance. Hence, the three crude extracts surpassed tylosin antibiotic, as well as they increased the workers brood rearing activity and the amounts of stored honey and pollen when compared with the control colonies. Perhaps, they enhanced the honey bees immune system. This suggests their suitability as antimicrobial and antioxidant agents in the control of other honey bee diseases and in food and drug industries. Further detailed studies are required to determine the constituents of the three herbal extracts and to evaluate their therapeutic applications.

INTRODUCTION

Honey bees health is a major current concern because of the substantial colony losses in recent years (vanEngelsdorp and Meixner, 2010). Infectious diseases are important factors affecting the development and consistency of honey bee colonies. The bacterial brood diseases are economically the most important worldwide as American Foulbrood (AFB) and European Foulbrood (EFB) (Genersch, 2010 and Morrissey *et al*., 2015). The causative agent of AFB is the Gram-positive bacterium *Paenibacillus larvae*. It forms highly contagious resistant and long-lived spores which can remain dormant for many years in the honey and combs. Hence, the disease extends by the exchange of infected combs among colonies, beekeeping tools or robber bees. Without periodical inspection by the beekeepers, the colony is very likely to be destroyed by the infection and even the whole apiary (Lodesani and Costa, 2005 and Genersch *et al.*, 2006).

The antibiotics oxytetracycline hydrochloride (OTC) and tylosin have a wide range of application in veterinary medicine and are applied for the treatment of bee

diseases (Mutinelli, 2003 and Adams *et al.*, 2007). However, the use of antibiotic risks persistence of chemical residues in honey and diminishing its quality for human consumption. Furthermore, the use of antibiotics may reduce the lifespan of honey bees (Albo *et al.*, 2003). Residues in honey are not allowed according to European legislation, but treatments with antibiotics are effected in many other countries (Bogdanov, 2006). The severe effects related to AFB and the complications associated with the application of antibiotics urge the researchers to develop alternative means for the disease control. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries (Antunez *et al.*, 2008 and Sharma and Bhadange, 2013). The use of essential oils or their specific volatile components may represent an alternative scope for the control of this serious disease without toxic chemical residues neither in honey bees nor in their by-products (Fuselli *et al.*, 2008). Herbal medicine represents one of the most important fields of traditional medicine all over the world. There has been growing interest in the investigation of the natural products from plants for the discovery of new antimicrobial agents (Edziri *et al*., 2011 and Ferreira *et al*., 2014). This work aimed to test the efficacy of three wild herbal crude extracts, which were used for the first time against American foulbrood disease (AFB) caused by *Paenibacillus larvae* in honeybee colonies.

MATERIALS AND METHODS

Plant extracts and antibiotic:

Three wild herbal plants were chosen according to the antimicrobial properties of their families to be tested against AFB. *Marrubium alysson* (Family: Labiatae; the aerial parts) was collected from Al-Arish, North Sinai province, Egypt (31°08'25.2"N & 33°50'10.7"E). *Lobularia arabica* (Family: Brassicaceae; the whole plant) and *Malcolmia pygmaea* (Family: Brassicaceae; the whole plant) were collected from Jabal Al-Narjes (daffodil hill), Baltim, Kafr El-Sheikh province, Egypt (31°35'41.3"N & 31°06'44.5"E). The plants were identified in the herbarium of Botany Department, Faculty of Science, Mansoura University, Dakahlia province, Egypt. Each herbal species was separately cleaned, washed and dried at room temperature. Thereafter, dried plants were grinded with an electric mill and sieved with a 0.5 mm sieve.

The extraction process was carried out according to Freedman *et al.* (1979) with minor modifications of a sequent soaking of the grinded plants in chosen solvents according to their polarity. The extraction solvents were petroleum ether, methylene chloride, ethyl acetate and methanol 70%. It was dissolved 1 g of each herbal methanolic extract in 100 ml of 80% methanol by shaking for 30 min protected from light. The resulting aqueous-methanol extract was filtered through a Whatman filter paper No. 1. This final hydroalcoholic solution was kept at 4°C until the antibacterial bioassay against *P. l. larvae*.

Mycovet is a commercial antibiotic tylosin used as veterinary oral water soluble powder (226 g of tylosin tartarate 100% equivalent to 200 g tylosin as an active ingredient). It has been registered under a number of 2450/2005 and was purchased from Copad Pharma Co., El Obour City, Industrial Zone, Block 12011, Cairo, Egypt.

Isolation and characterization of *P. l. larvae*

AFB disease was diagnosed in the field according to the criteria of Shimanuki and Knox (2000). Infected larvae and comb pieces (20 cm^2), were collected from

severely AFB-infected apiaries. The samples were kept at 4°C for isolation and diagnosis of the bacterial contagion (Owayss, 2007). Dead and diseased larvae were crushed in 0.5% sterile peptone, heated at 80°C for 10 min to kill non-spore-forming bacteria. Then, it was centrifuged at 6000 rpm for 45 min and the pellet containing the bacterial spores was used for surface inoculation. The bacterial growth medium was Brain Heart Infusion Agar (Difco) fortified with 0.1 g thiamine hydrochloride (sterilized by Millipore filter paper) per liter of sterilized medium (BHIT) and adjusted to pH 6.6 with HCl. The medium was autoclaved at 10 lb/sq. at 116°C for 20 min and poured into 90-mm diameter Petri dishes, 20 ml/plate, which were incubated at 35°C for 72 hr (Shimanuki and Knox, 1991). Emerged colonies were picked on BHIT media and the bacterial isolates were purified by repeated streaking on the respective medium and Gram-stained. Gram-positive, spore-forming rod-shaped isolates were subjected to microscopic examination. One colony from each characterized bacterial isolate was subjected to molecular identification using the 16S rRNA gene. The PCR reaction was performed according to Joshi and Deshpande *et al*. (2010) to complete identification of *P. l. larvae*.

Safety test

Toxicity effect of the plant extracts on the Carniolan hybrid race was evaluated using a technique developed by (Maggi *et al*., 2010). Adult worker bees were collected from the brood area of the healthy colonies and transported into special breeding cages with one wire mesh covered side (16 cm × 12 cm × 6 cm) as 300 worker bees/cage. The control group was administered only sugar syrup (2w : 1v). For each herbal crude extract, 10 ml of sugar syrup were blended to result in final concentrations of 1000, 750 and 500 ppm. The honey bee workers were fed *ad libitum* via a piece of cotton impregnated with the tested solution. All cages are triplicated and incubated at 28 ± 2°C and 65% RH for 72 h. Dead workers were daily counted and discarded. Finally, bees were sacrificed and the mortality percentages were calculated.

Artificial infection with AFB

The spore suspension of *P. l. larvae* was prepared according to the method of Shimanuki and Knox (1991). The spore suspension was inoculated by spraying on the honeybee combs in the tested colonies (50 ml/colony). The disease progress was evaluated by weekly inspection and infected larvae were counted per colony (Hitchcock *et al.*, 1970). The American foulbrood disease symptoms were diagnosed according to Shimanuki and Knox (2000).

Experimental design

The experiment was carried out in an outdoor apiary at Shoha village, Mansoura city, Dakahlia province, Egypt (31°04'39.7"N and 31°28'46.4"E) during the period from March to September 2014. Eighteen healthy honeybee colonies of Carniolan hybrid race in typical one-chambered Langstroth hives with sufficient brood and food were chosen. Each colony was headed with a new and very active egg-laying queen. Efforts were also made to prevent dequeening and control swarming. The colonies were categorized into six groups, three colonies for each as replicates. Group (1) was control and fed only sugar syrup (1w: 1v). Group (2) was inoculated only with *P. l. larvae*. Group (3) was inoculated with *P. l. larvae* and treated with 200 mg tylosin antibiotic solved in 250 ml of sugar syrup (1w: 1v). While, group (4), (5) and (6) were inoculated with *P. l. larvae* and treated with *Marrubium alysson*, *Lobularia arabica* and *Malcolmia pygmaea* crude extracts,

respectively. Each herbal extract was added to 250 ml of sugar syrup (1w : 1v) as 1000, 750 and 500 ppm, which were sequentially applied like tylosin at one-week interval. The first treatments were carried out at the same day of the disease symptoms appearance.

Based on the method of Nour (1992), some biological activities were measured as population biomarkers to explain the effect of the artificial infection and treatments on the honeybee groups in comparison with the control one. Unsealed and sealed workers brood rearing activity and the amounts of stored honey and pollen were measured in sq. in/colony at a 12-day interval. Estimation of honey as kg/colony was calculated according to Shawer *et al.* (1986) and pollen as g/colony was calculated according to Ismail (2006).

Statistical analysis

The data were checked for normality and homogeneity of variance with Klomogrov-Smirnov and Levene tests, respectively. Parametric data were analyzed with One-Way ANOVA for analysis of variance followed by honest Tukey test for post-comparison of significant difference between groups. Non-parametric data were tested with Kruskal-Wallis test followed by Mann-Whitney (U) test. For correlation coefficient, Pearson and Kendall tau-b tests were used for parametric and non-parametric data, respectively. Statistical significance difference was accepted at $p < 0.05$ with double-sided type (two-tailed) distribution. All data were statistically manipulated using SPSS Statistics program 17.0© 1993-2007.

RESULTS

Safety of the herbal extracts

After 24 h, there were no dead larvae were found in the cages of the control group and all concentrations of the herbal extracts, while after 48 h, a few number of dead workers were recorded. The highest number was recorded after 72 hrs, particularly, for 1000 ppm concentration followed by 750 ppm. However, the number of dead bees was considered low in all treatments. Mortality percentages ranged from 2.07 to 3.67% for the used concentrations of the three herbal extracts with insignificant differences when compared with the control cages except for 1000 ppm concentration (Fig. 1).

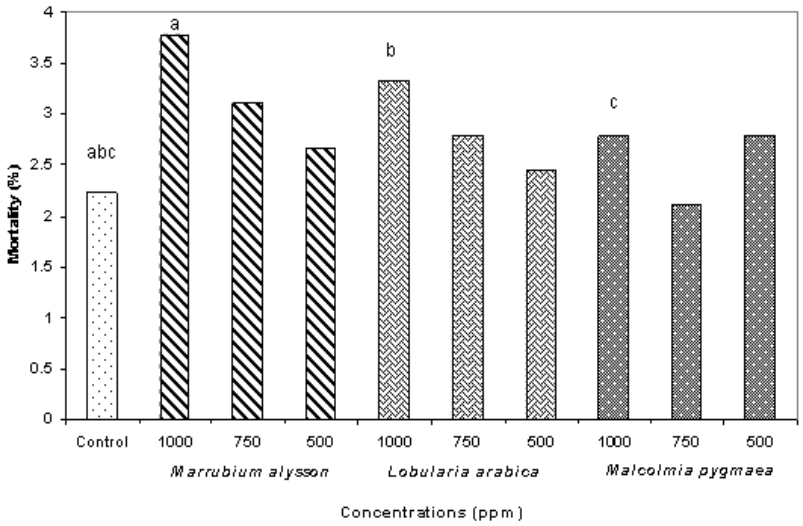

Fig. 1: Mortality of the adult honey bee workers treated with the three herbal extracts (n = 300) and the same letter means significant different.

Incidence of American foulbrood

The control colonies did not exhibit any symptoms of the disease during the experiment. No infection was recorded in all groups during March and April. On May 1, after 3 weeks from the inoculation, the first appearance of symptoms was recorded in the infected and treated colonies and mainly constricted in May. Tylosin-treated, infected and *Lobularia arabica* extract-treated groups had significant increases with averages of 27.0, 26.78 and 23.0 infected larvae/colony, respectively when compared with other groups. *Marrubium alysson* extract- and *Malcolmia pygmaea* extract-treated groups had averages of 17.22 and 14.33 infected larvae/colony, respectively. In August, a slight infection reappeared again in tylosin-treated colonies only with an average of 1.17 infected larvae/colony (Fig. 2). The total means of the infected larvae significantly increased in the tylosin-treated and infected groups in comparison with the control group with values of 5.95 and 5.74 infected larvae/colony, respectively. There are insignificant increases in *Lobularia arabica*-, *Marrubium alysson*- and *Malcolmia pygmaea*-treated colonies when compared with the control one with total means of 4.93, 3.69 and 3.07 infected larvae/colony, respectively (Fig. 3).

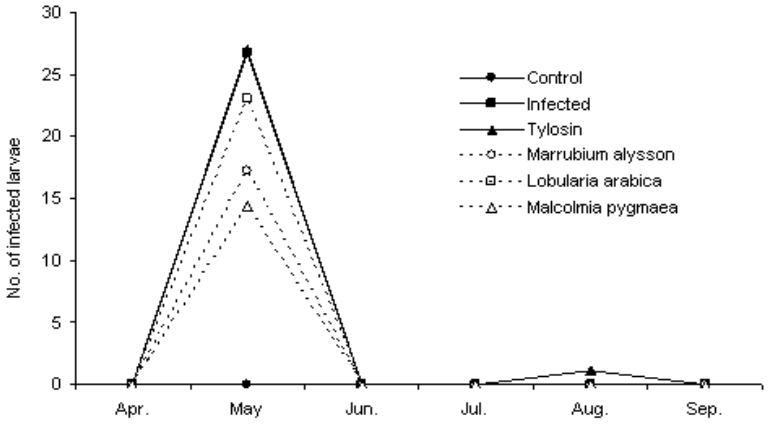

Fig. 2: Monthly average numbers of larvae infected with AFB in the different experimental groups.

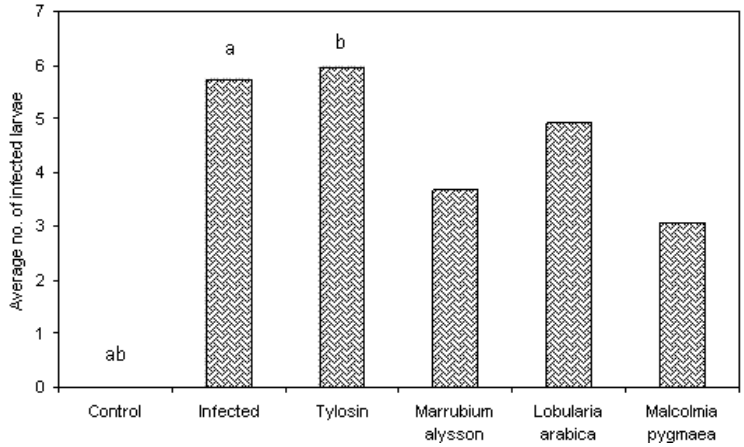

Fig. 3: Total average numbers of larvae infected with AFB in the different experimental groups (the same letters mean significant differences).

Biological activities

Sealed workers brood rearing activity

In April, *Marrubium alysson*-treated colonies exhibited the highest sealed workers brood area with an average of 710.67 sq. in/colony. The amounts greatly

increased in May with a climax and *Malcolmia pygmaea*-treated colonies reared the biggest sealed workers brood area with an average of 1143.80 sq. in/colony. Thereafter, the amounts greatly decreased in June until September with a slight increase in August and the highest average was 599.35 sq. in/colony in *Lobularia arabica*-treated colonies. (Fig. 4). The highest total averages were recorded in infected and *Marrubium alysson*-treated groups with values of 578.55 and 574.70 sq. in/colony. The lowest total mean was recorded in tylosin-treated group with a significant decrease of 448.72 sq. in/colony when compared with *Marrubium alysson*-treated and infected groups. Otherwise, there were insignificant differences for control, *Malcolmia pygmaea* and *Lobularia arabica*-treated groups (Fig. 5).

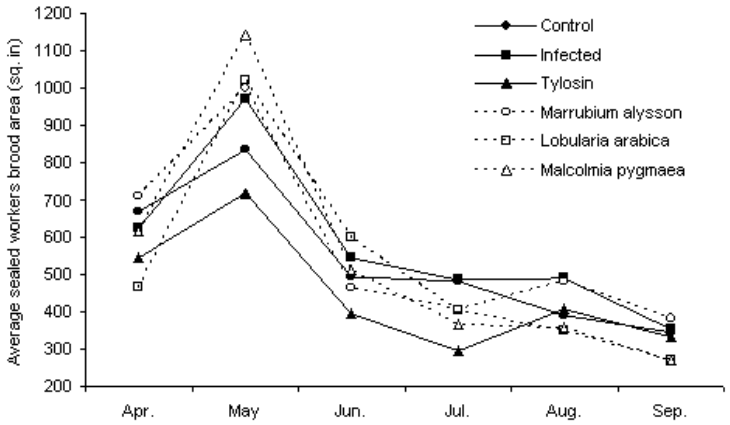

Fig. 4: Monthly averages of sealed worker brood areas reared by the different experimental groups.

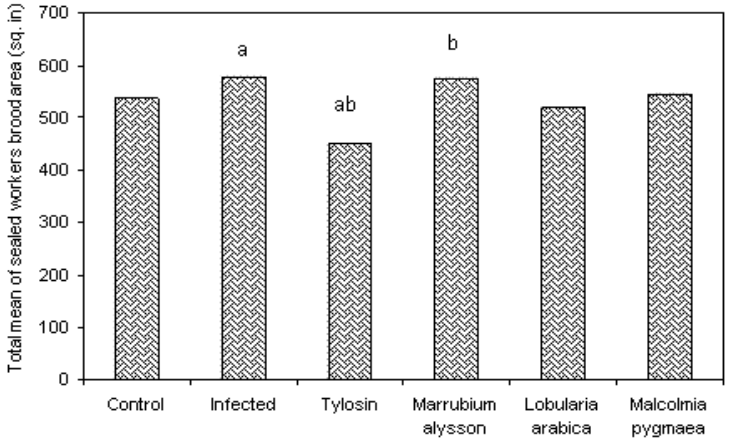

Fig. 5: Total averages of sealed worker brood areas reared by the different experimental groups (the same letters mean significant differences).

Honey storage activity

Malcolmia pygmaea-treated colonies stored the highest amounts of capped honey during the experiment followed by *Marrubium alysson*-, *Lobularia arabica*- and tylosin-treated colonies. The average stored amounts by *Malcolmia pygmaea*-treated colonies were 3.34, 10.47, 13.54, 12.25, 6.26 and 2.00 kg/colony in April, May, June, July, August and September, respectively. The lowest amounts were recorded in control and infected groups with significant decreases when compared with the other groups (Fig. 6). The total mean values of capped honey were 7.98, 6.82, 6.19, 5.36, 3.59 and 3.36 kg/colony stored by *Malcolmia pygmaea*-, *Marrubium alysson*-, *Lobularia arabica*-, tylosin-treated, control and infected colonies,

respectively. There were significant increases in the stored honey amounts by the three herbal extract-treated colonies in comparison with the control and infected colonies (Fig. 7).

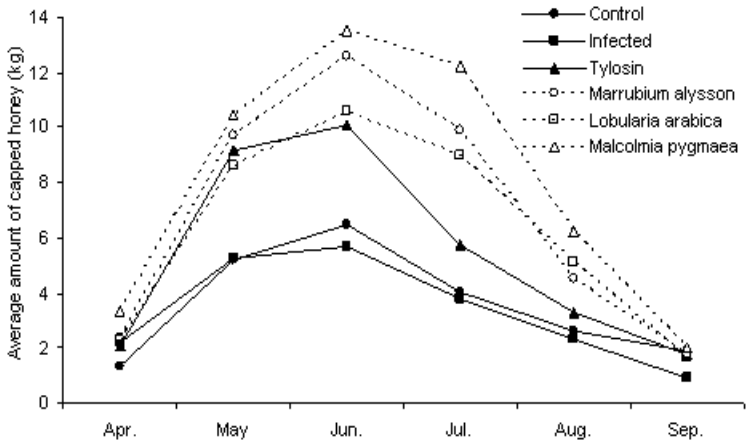

Fig. 6: Monthly averages amounts of capped honey stored by honey bees of the different experimental groups.

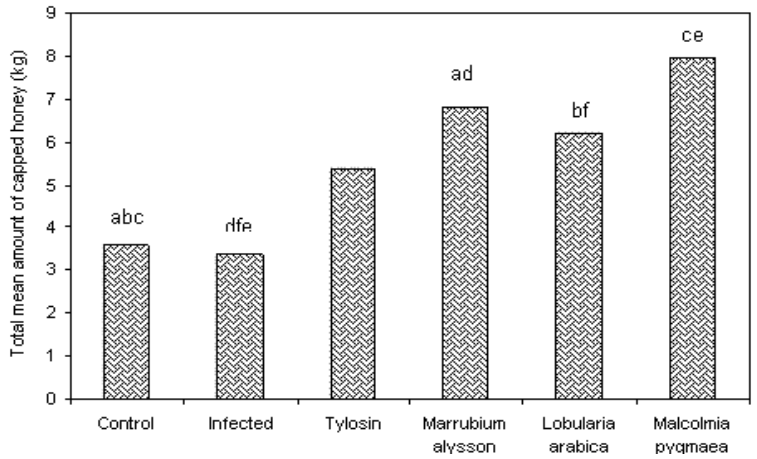

Fig. 7: Total averages amounts of capped honey stored by honey bees of the different experimental groups (the same letters mean significant differences).

Pollen storage activity

In April, the stored pollen amounts were greatly small and the highest average was 144.59 g/colony for *Malcolmia pygmaea*-treated colonies. In May, the amounts greatly increased and were the maximum in *Marrubium alysson*-treated colonies with an average of 1079.61 g/colony. In June, the pollen storage activity reached its climax and *Lobularia arabica*-treated colonies stored the largest amounts of pollen with an average of 1232.36 g/colony. In July, the amounts decreased and the largest was recorded from the *Marrubium alysson*-treated colonies with an average of 926.13 g/colony. In August, the largest recorded amount of pollen was 535.75 g/colony in infected colonies. In September, the stored amounts of pollen greatly decreased and tylosin-treated colonies was the highest with an average of 237.13 g/colony (Fig. 8). There were insignificant differences among all the experimental groups. The highest amounts of stored pollen was recorded from *Marrubium alysson*-treated group with a total mean of 644.71 g/colony followed by *Lobularia arabica*- and tylosin-treated groups with total means of 616.15 and 615.76 g/colony, respectively. The lowest

amounts recorded from *Malcolmia pygmaea*-treated, control and infected groups with total means of 568.98, 562.44 and 492.57 g/colony, respectively (Fig. 9).

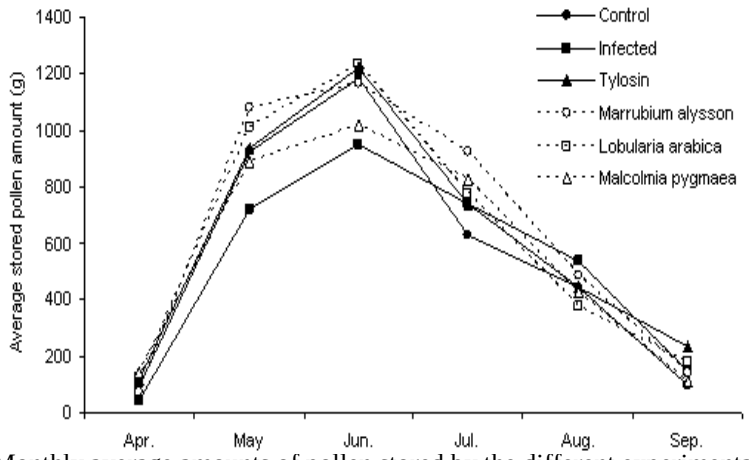

Fig. 8: Monthly average amounts of pollen stored by the different experimental groups.

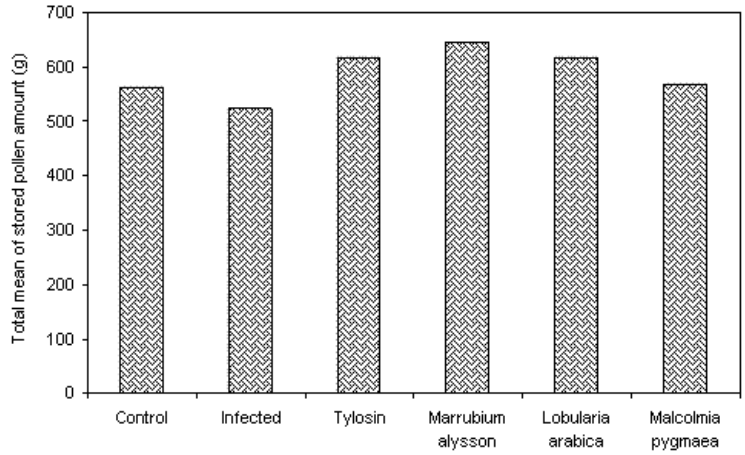

Fig. 9: Total average amounts of pollen stored by the different experimental groups.

DISCUSSION

The present study included the laboratory safety tests of the three herbal extracts against honey bee workers. The mortality percentages were less than 4% as the normal range for all concentrations. Thus, the crude extracts did not exceed the toxicity limits and are considered safe for honey bees population in management of American foulbrood disease. This agreed with Flesar *et al*. (2010) who showed that the most active natural products of some tested plant extracts were non-toxic at concentrations as high as 100 µg per adult bee. After artificial inoculation and with weekly inspection, the AFB disease appeared in the treated cells and diagnosed according to Shimanuki and Knox (2000). The irregular sunken or spotty sealed workers brood like pepper box with darker capping are the typifying of AFB disease. Infected larvae turned concave, colored and extended lengthwise in the cell. The dead larvae exhibited a "ropy" condition that can be demonstrated by inserting a matchstick and drawing out, giving a fine elastic threadlike projection from 2.5-3 cm.

It was observed the increase in sealed workers brood rearing activities, as well as stored honey and pollen amounts in spring (clover season) when compared with summer (maize season). The current results go on the same line with the findings

recorded by Abou-Elenean (2002); Shawer *et al.* (2003); Taha (2005) and Abd Al-Hady (2007). They reported that, for F1 Carniolan race, the largest amount of workers brood was reared through spring followed by summer. Two peaks of worker brood appeared through clover and cotton nectar flow. Serag El-Dein (2004) reported that in Kafr El-Sheikh region, the highest averages of sealed worker brood areas was found during May and July, while the lowest area was recorded during November. On the other hand, in Assiut governorate, Abdel-Rahman (1998) recorded that the maximum monthly averages of sealed workers brood areas during September, July and February, while the minimum areas were noticed during December and November. Under environmental conditions of Giza governorate, Zeedan (2002) reported that the maximum worker brood amounts, for F1 Carniolan race colonies, were achieved during summer season. Mansour *et al.* (2003) indicated that, in Kafr El-Sheikh governorate, the largest area of sealed brood was obtained during summer.

In this study, the amounts of stored (capped) honey could be compared to those recorded by Abou-Elenean (2002) for the Carniolan race. Most of the harvested honey was during clover season in June and during cotton season in September. Helal *et al.* (2003) stated that the largest yield of honey was collected during May (clover season), followed by April (citrus blooming period). Abd Al-Hady (2007) reported that the highest rate of honey storage for F1 Carniolan race colonies was during spring followed by summer, autumn and winter. This is in contrast with the largest average honey yield was obtained from cotton in July followed by clover in May in Assiut governorate (Abdel-Rahman, 2004). Also, it was mentioned that the highest mean of honey yield was obtained during cotton nectar flow in Biala, Kafr El-Sheikh governorate (Serag El-Dein, 2004).

The highest rates of pollen storage activity extended from May to August and this coincided with the results of Sharaf (1996) in El-Behira governorate. Also, the same was recorded by Abo El-Kassem (1997) and Shawer *et al.* (2003) who reported that the largest stored pollen areas, for F1 Carniolan race colonies, was during clover and cotton seasons at Kafr El-Sheikh governorate. Serag El-Dein (2004) indicated that the largest amounts of pollen, for F1 Carniolan race colonies, were gathered during clover nectar flow, followed by maize and broad bean in Biala, Kafr El-Sheikh governorate. El-Sherif *et al.* (1994) recorded longer period of the ample pollen storage activity in El-Kanater El-Khayria, Qalubia governorate from mid-March to mid-September. Taha (2005) estimated greatly smaller amounts of stored pollen during clover and faba bean blooming periods in Motobes, Kafr El-Sheikh governorate.

This study showed that tylosin antibiotic was the least effective in controlling the American foulbrood disease when compared with the herbal crude extracts. The workers brood rearing and honey storage activities greatly decreased due to tylosin application. Moreover, the infection reappeared again only in tylosin-treated colonies in August, indicating to the resistance of the bacterial causative agent to the antibiotics. Drilca (2003) reported that the mutation potential, the genetic interchange between bacteria and the wrong therapeutic application can rapidly produce resistant populations. The efficacy of tylosin has already been proven through the use of different application methods (Alippi *et al*., 2005 and Reynaldi *et al*., 2009). This feature makes tylosin an alternative for the control of infected hives with oxytetracycline-resistant isolates. Thus, several honey producers countries as Argentina, Canada, China and USA allow the use of antibiotics to keep the disease under control (Reybroeck *et al*., 2012).

Malcolmia pygmaea methanolic extract exhibited the highest antibacterial activity and was the most effective against the AFB disease in Carniolan colonies. Although the herbal extracts are common as folk remedies due to their antimicrobial and antioxidant properties, the publications of the three herbal species are very scarce. Esmaeili *et al*. (2014a) showed that the essential oils of the aerial and seed parts of *Malcolmia africana* (Cruciferae) contain α-Eudesmol (12.4%), isothiocyanato ethane (11.5%), limonene (10.3%), and jasmone (8.8%). Esmaeili *et al*. (2014b) proved that the methanolic extract of *Malcolmia africana* aerial and seed parts had the highest amounts of phenolics, which exhibited a moderate antibacterial activities against seven Gram-positive and Gram-negative bacteria. Jahgangir *et al*. (2009) stated that numerous members of Brassicaceae have been commercialized globally as a rich source of nutrients and healthy products for animal and human consumption. Dimayuga *et al*. (1991) and Prasad (2014) showed that Cruciferae is rich in polyphenols, flavonoids, and glucosinolates and their hydrolysis products have proved to have antibacterial, antioxidant and anticancer properties.

Marrubium alysson methanolic extract was lower than *Malcolmia pygmaea* in its efficacy of AFB control but there were insignificant differences between them in workers brood rearing and honey storage activities. Methanolic extract of the leaves of *Marrubium alysson* has the most potent antiviral activity against human cytomegalovirus (HCMV) strain *in vitro* (Edziri *et al*., 2011). *Marrubium vulgare* exhibited antibacterial, antifungal and cytotoxic properties (Zarai *et al*., 2011 and Rigi *et al*., 2013). The methanolic extract of *Marrubium peregrinum* exhibited potential antibacterial and antifungal activities at concentrations could be comparable to those of this work (Radojević *et al*., 2013). It was discovered 86 kinds of compounds in *Marrubium anisodan* extract mainly contained furfural, steroids, vitamin B and flavonoids with antifungal activities (Mohammadi and Piri, 2014). *Marrubium alysson* belongs to family Labiatae (Lamiaceae) that their plants are known significantly not only for their aroma but also for their antimicrobial and medicinal properties (Sharma and Bhadange, 2013). *Lobularia arabica* methanolic extract was the least effective in AFB control and on the population parameters of the Carniolan honey bees among the three herbal extracts. Al-Gendy *et al*. (2016) identified three glucosinolates from the genus *Lobularia lybica* with antimicrobial and cytotoxic activities.

The hygienic behavior as a defense mechanism against diseases may aid along with the herbal extracts in the disappearance of AFB symptoms in most months (Abdel-Wahab, 2001). The improvement of the honey bee biological activities indicated to the crude herbal extracts may increase the immune-system of the honeybee larvae against the AFB disease (Zakaria, 2007). Recently, uncontrolled use of antibiotics created antibiotic resistance, which focuses attention towards the discovering of new herbal drugs. Nature is a mine of herbal phytochemicals which are entrapped in green treasure of earth as the medicinal and aromatic plants (Sharma and Bhadange, 2013). The plant extracts may be used against AFB because they are available, cheap, easy to apply and have no chemical toxic residues in bee products (Flesar *et al*., 2010). In a recent study, the total phenolics, flavonoids and tannins of the aqueous and methanolic extracts of siwak and cinnamon had significant antibacterial effects against *Paenibacillus larvae* (Hashish *et al*., 2016).

CONCLUSION

The present findings indicates to that the crude methanolic extracts of all the three tested wild herbs have antibacterial properties against the American foulbrood disease. *Malcolmia pygmaea* is the most potent followed by *Marrubium alysson* and *Lobularia arabica*, which is the least effective extract. All of them are safe to adult honey bee workers so that they increase the brood rearing activity and the amounts of stored honey and pollen when compared with the control colonies. The herbal extracts may enhance the honey bees immune system, as well as their cheapness and availability. This suggests their suitability as antimicrobial and antioxidative agents in the food and drug industries. These findings revealed that these herbal medicinal plants are promising as AFB-control agents. The chemical constituents should be identified to be tested against the disease with further detailed studies to evaluate their therapeutic applications.

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ARABIC SUMMERY

دور بعض المستخلصات العشبية البرية فى السيطرة على مرض تعفن الحضنة الأمريكى فى طوائف نحل العسل

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يعتبر مرض عفن الحضنة الأمريكى واحد من أهم الممرضات البكتيرية التي تصيب نحل العسل مسببة خسائر كبرى لقطاع تربية النحل على الصعيد العالمي. وتعتبر جرثومات البكتريا العصوية *Peanibacillus larvae* هى السبب الرئيسى فى عدوى يرقات النحل بهذا المرض. تناولت هذه الدراسة تأثير المستخلصات الميثانولية الخام لثلاث من النباتات العشبية البرية التى تنمو فى مصر على مرض الحضنة الأمريكى فى خلايا النحل بعد استحداث العدوى فيها مع استخدام المضاد الحيوى التيلوزين كعقار للمقارنة. أثبتت التجارب المعملية الخواص الآمنة لهذه المستخلصات الثلاثة على شغالات النحل البالغة. كانت عشبة الشلوة القزمة *Malcolmia pygmaea* هى الأكثر فاعلية ضد المرض وتتبع الفصيلة الصليبية وتلتها عشبة فراسيون أليسون *Marrubium alysson* وتتبع الفصيلة الشفوية وكانت عشبة خرم الإبرة العربى *Lobularia arabica* هى الأقل تأثيراً بينها جميعا. كان المضاد الحيوى التيلوزين الأضعف تجاه المرض مما يشير لتنامى المناعة البكتيرية ضده، ولذلك فاقت المستخلصات العشبية الثلاث فى كفاءتها المضاد الحيوى، بالإضافة لتأثيرها الواضح على زيادة أنشطة تربية حضنة الشغالات المغلقة وتخزين العسل واللقاح فى الخلايا مقارنة بخلايا المجموعة الضابطة مما يشير للتأثير الإيجابى على مناعة النحل وبيولوجيته بصفة عامة. فاعلية المستخلصات الخام الثلاثة ضد المرض وتأثيرها الآمن والإيجابى على أنشطة النحل يسفر عن كون هذه الأعشاب البرية تحتوى على مكونات ذات تأثير ضد ميكروبى عال ومضادات للأكسدة مما يرجح إمكانية الحصول على عقاقير ذات كفاءة عالية فى مكافحة مرض تعفن الحضنة الأمريكى وربما بعض أمراض النحل الأخرى، ويرشح هذه النباتات كعنصر هام فى الصناعات الغذائية والدوائية. لذلك توجب استكمال البحث على هذه المستخلصات الخام لمعرفة المكونات الرئيسية بدقة والتأثير العلاجى لكل منها وهذا يحتاج لدراسات مستفيضة لاحقا.