

Studies on Brood diseases in honey bee

Fatma Taha Ahmed El-Salhy

Studies on brood diseases in honeybees The honeybee, *Apis mellifera* L. is considered one of the most important beneficial insects for the man. Like all living organisms, honeybees can be infested with diseases. It is important for the beekeeper to be able to recognize conditions that might be disease related and respond accordingly. The bees have two distinct life forms (brood and adult) and most diseases are specific to either one stage or the other. The most virulent diseases at present are those of the brood specifically Varroa disease, American foulbrood and European foulbrood. Other brood diseases include chalk brood, a fungal disease that appears to be on the rise and sac brood caused by a virus. These studies were carried out to determine the damage of honeybee brood diseases, contained the serious disease named varroasis and its suitable control methods. Obtained results could be summarized as follows:

1- The damage:

a- The effect of Varroa infestation on the body weight and longevity of newly emerged honeybee workers. The body weight of newly emerged workers were greatly reduced due to varroa infestation. The mean weight of newly emerged healthy workers recorded 114.210, 118.660 and 115.520 mg, for Carniolan, F1 Carniolan and F1 Italian hybrids respectively. Whereas the mean weight of infested workers were 100.000, 104.00 and 105.100 mg when the infestation level was 1 mite/ worker pupae; 74.100, 77.33 and 78.66 mg with the highest intensity of infestation for Carniolan race, F1 Carniolan and F1 Italian hybrids, respectively.

b- The longevity of emerged workers: The longevity were 25.320, 26.960 and 29.210 days for healthy emerged workers of Carniolan race, F1 Carniolan and F1 Italian hybrids, respectively. When the level of varroa infestation was 1 mite/ worker pupae, the mean longevity were 12.166, 13.450 and 15.360 days for the three respective bee race and hybrids, whereas the longevity of infested emerged workers were 6.315, 7.600 and 8.550 days for Carniolan race, F1 Carniolan and F1 Italian hybrids when the intensity of infestation was the maximum. Statistical analysis revealed a significant negative correlation between the weight of infested bees and the intensity (level) of infestation and so in that of longevity in emerged honeybee workers infested with varroa mite. The rate of weight loss due to varroa infestation ranged between 12.441 — 35.119, 12.354 — 34.830 and 9.020 — 31.907% for workers belong to Carniolan race, F1 Carniolan and F1 Italian hybrids, respectively. The respective ranges for longevity were 51.951 — 75.059, 50.111 — 71.810 and 47.415 — 70.729%.

2- Effect of varroa infestation on the mortality rate of newly emerged honeybee workers. The percent increase of mortality in emerged workers of Carniolan race was 78% after 6 days from the emergence when the intensity of infestation was more than 2 mites/ worker pupa. F1 Carniolan emergence worker intermediate (60% mortality) after 8 days from the emerging. F1 Italian proved less susceptibility recording 59% mortality after 10 days from emerging. It is obvious that varroa infestation during pupal stage increased greatly the percent mortality of the resulted workers. The increase in workers mortality was highly significant.

3- Susceptibility of emerged honeybee workers to varroa mite infestation. Obtained results showed clearly that the time was passed until 50% emerged honeybee workers had died (LT50) were 4, 6 and 8 days when the intensity of infestation were 2 mite/ worker pupa for Carniolan race, F, Carniolan and F, Italian hybrids, respectively. In the same time (LT90) recorded 6, 8 and 10 days for the mentioned honey bee race and hybrids. When the intensity of infestation was more than 2 mites/ worker pupa Carniolan race emerged worker proved the most susceptible as mentioned before recording 2 days and 4 days for (LT50) and (LT 90), respectively- whereas emerged workers of F, Carniolan hybrids was intermediate recording 4 and 6 days for (LT50) and (LT90), respectively. As previous F, Italian

recorded 6 and 8 days for (LT50) and (LT90), respectively which it proved the least susceptible to varroa infestation.

4-Effect of varroa infestation on malformations of newly emerged honeybees

The deformation of newly emerged bees (workers and drones) positive correlated with the number of varroa mites/ cell. The obtained results showed that the deformed bees were appeared when the intensity of varroa infestation was 3 mites/ pupae. The bees of Carniolan race were more affected with varroa infestation (deformed % 28.571, 83.333%) and (43.750, 90.00%) than both F, Carniolan (26.315, 80.00%) and (41.176, 85.714%) and F, Italian (21.428, 75.00%) and (37.500, 83.333%) for emerged workers and drones when the intensity of infestation were 3, 6 mites/ cell pupae, respectively.

5-Biometrical studies for the effects of varroa mite infestation on certain morphological, aspects of honeybee workers.

The reduction percentage due to the effect of varroa mite infestation on certain morphological characters of Carniolan race, F1 Carniolan and F1 Italian newly emerged honeybee workers when the intensity of varroa infestation was more than 3 mite/ worker pupa as follow: Proboscis length 25.251%, 19.095% and 14.157%, respectively; fore — wing length 21.538%, 11.538% and 7.608%, respectively; for — wing width 34.887%, 24.185% and 14.572%, respectively; Hind — tibia length 13.425%, 17.445% and 10.691%, respectively; Hind — basitarsus length 11.893%, 9.353% and 9.645%, respectively; Hind — basitarsus width 12.596%, 10.128% and 10.297%, respectively; Hind femur length 5.686%, 4.980% and 2.742%, respectively; pollen basket length 14.705%, 13.117% and 13.412%, respectively and pollen basket width 39.118%, 37.433% and 30.136%, respectively. It is obvious that F1 Italian hybrids showed the least reduction percentage in the mentioned morphological characters affected with varroa mite infestation and the F1 Carniolan hybrids intermediate in that case. On the other hand Carniolan race proved to be the highest sensitive to varroa infestation which recorded the highest reduction percentage in that morphological characters were mentioned.

II-Efficiency of control agents against Varroa mite.

1-Effect of some control agents on the infestation and number of fallen mites.

a-Percent reduction in rate of infestation

Carniolan race proved to be the most susceptible race to the mite infestation manifesting infestation range between 22.33 — 32.33% (average 27.037%). F1 Carniolan hybrids was intermediate in this respect, as its infestation range was between 18.66 — 27.66% (average 23.411%). whereas F1 Italian hybrid proved to be the least susceptible hybrid, as its infestation range was 16.33 — 27.00% (average 20.805%). The test control agents could be arranged descendingly to the efficiency in reducing the rate of varroa infestation in Carniolan race as follow: formic acid 85% with daily evaporation rates, 0.9 cm³ (94.9%), 1.0 cm³ + camphor 0.5 tablet/ month (89.1%), 1.1 cm³ (82.4%), 1.0 tablet/ 1.5 months (74.7%), 1.3 cm³ (65.7%), 0.5 tablet/ month (61.5%) and 1.0 tablet/ month (60.8%). For F1 Carniolan, formic acid 85% with daily evaporation rates 1.3 cm³ (efficiency 93.5%), 1.1 cm³ (efficiency 88.3%), 1.0 cm³ + Camphor 0.5 tablet/ month (efficiency 84.3%), 0.9 cm³ (efficiency 76.7%), Camphor 1.0 tablet/ month (efficiency 69.7%), 1.0 tablet/ 1.5 month (efficiency 63.6%) and 0.5 tablet/ month (efficiency 56.7%). For F, Italian, Camphor 1.0 tablet/ month (efficiency 93.5%), formic acid 85% with daily evaporation rates 1.1 cm³ (efficiency 88.1%), 1.3 cm³ (efficiency 87.8%), 1.0 cm³ + Camphor 0.5 tablet/ month (efficiency 78.2%), 0.9 cm³ (efficiency 69.2%), Camphor 1.0 tablet/ 1.5 month (efficiency 65.4%) and Camphor 0.5 tablet/ month (efficiency 64.7%).

b-Number of fallen mites

The highest numbers of fallen mites in all treatments were recorded at 12 days from the onset of the application then decreased gradually as time lapsed. However, the highest number were counted with the suitable control agent in Carniolan race, F1 Carniolan and F1 Italian hybrids when the colonies were exposed to formic acid 85% with daily evaporation rate 0.9 cm³ (4720.7 mites/ colony), 1.3 cm³ (4735.6 mites/ colony) and Camphor 1.0 tablet/ month (3822.7 mites/ colony).

2-Effect of some control agents on brood rearing activity.

The data concerning the effect of varroa infestation on brood rearing activity and the subsequent effect of control agents on this parameter revealed the following remarks:

a-As for the total brood area reared of Carniolan race during the eight counts, the test control agents could be arranged descendingly as follows: formic acid 85% with daily evaporation rates 0.9 cm³ (926 inch²), 1.1 cm³ (873 inch²) and 1.3 cm³ (870 inch²), 1.0 cm³ + Camphor 0.5 tablet/ month (863 inch²), Camphor with doses 0.5 tablet/ month (779 inch²), 1.0 tablet/ 1.5 months (714 inch²) and 1.0 tablet/ month (578 inch²).

b-The test control agents could be arranged descendingly according to their efficiency in inducing brood rearing activity in F1 Carniolan hybrids (referred to as percent increase

in brood area compared to that of control colonies) as follows : formic acid 85% with daily evaporation rates 0.9 cm³ (2138.71%), 1.1 294cm³ (2031.75%), 1.3 cm³ (1936.23%) and 1.0 cm³ + Camphor 0.5 tablet/ month (1880.59%), Camphor with doses 1.0 tablet/ month (1778.33%), 1.0 tablet/ 1.5 months (1580.70%) and 0.5 tablet/ month (1442.37%).c-The test control agents could be arranged descendingly according to the rate of increase in brood area of F1 Italaian hybrids when compared to untreated of that hybrid as follow: Camphor 1.0 tablet/ month (2884.6%), formic acid 85% with daily evaporation rates 1.3 cm³ (2116.9%), 0.9 cm³ (2049.0%), 1.1 cm³ (1924.1%) and 1.0 cm³ + Camphor 0.5 tablet/ month (1788.2%), Camphor with doses 1.0 tablet/ 1.5 months (1674.0%) and 0.5 tablet/ month (1378.7%) .d-F1 Caniolan proved to be the most active in brood rearing activity (average 1138.62 inch²). whereas F1 Italian colonies were intermediate (averaged 1004.00 inch²). Carniolan race colonies showed the lowest brood rearing activity (averaged 767.37 inch²).3-Effect of some control agents on honey yield production .All the test control agents applied to control Varroa mite induced significantly yield honey production in treated colonies when compared to the honey yield of untreated colonies belong to Carniolan race, F1 Caniolan and F1 Italian colonies. Generally, Generally, F1 Italian colonies showed the highest response to the control agents, as the honey yield in treated colonies, was markedly higher, recording a mean of 9.492 kg/ colony as compared to untreated colonies of the same hybrid. On the other hand, Carniolan colonies showed the lowest response, recording 7.514 kg/ colony in honey yield when compared with that ofuntreated colonies. However, F1 Caniolan colonies were intermediate, recording 8.371 kg! colony in honey yield more than control colonies.Formic acid treatments with daily evaporation rates 1.3 cm³, 1.1 cm³, 0.9 cm³ and 1.0 cm³ + Camphor 0.5 tablet/ month induced the highest honey production. The four treatments recorded percent increase of 1218.086%, 1318.478%, 1181.130% and 1175.000% in honey yield, in general , as compared to the yield harvested from untreated colonies, respectively, regardless of bee race. On the other hand, Camphor with doses 1.0 tablet/ 1.5 months and 0.5 tablet/ month treatments resulted in the lowest honey yield among the test agents, recording 505.782% and 478.260% increase more than that of untreated colonies, respectively. whereas, Camphor 1.0 tablet/ month were intermediate recording 1147.086%.4-Effect of oxalic acid with some control agents against Varroa mite.Oxalic acid 4.0% concentration proved to be higher in efficiency percent effect (93.333%) than 3.0% concentration which recorded (75.669%). whereas, 2.0 concentration showed lower efficiency effect (59.380%) but it was the lowest adverse effect on killing bees. Therefore, and if necessary 2.0% oxalic is recommended as it has efficiency in fallen mites, more safe (low pollution), lower effect in killed bees and less expensive.The highest significant mean number of fallen mites every week during the tested period (42 days) was recorded in bee colonies treated with (A) compound that were 380.00 mites/ colony. On the other hand, (B) compound treatment resulted in the lowest mean number of fallen mites (210.00 mites/ colony).Control colonies recorded only 34.00 mites/ colony. Statistical analysis revealed high significant differences between compound (A), (B) and the other treatments. The two compounds (A) and (B) could be arranged descendingly according to the percent reduction of infestation (efficiency) compared to control colonies as follows: Compound A (90.022%) and compound B (85.744%).5-Effect of Moshtohor compound (C) against Varroa mite.The different methods of compound C applications as a control agent could be arranged descendingly according to percent reduction of Varroa mite infestation (efficiency) as follow: 10.0 gin inserted between brood combs/ colony (84.427%), 10.0 gin on top bars/ colony (82.847%) 1.0 gm/ colony every week — sprayed 6 times in sugar solution (75.879%) 5.0 gm on top bars/ colony (64.308%) and 0.5 gm/ colony every week — sprayed 6 times in sugar solution (60.835%). In addition, the method applied of 10.0 gm inserted between brood combs/ colony proved to be the most effective control method of compound C as it reduced the percent of Varroa infestation (efficiency) by 84.427% within 42 days of application in test colonies.In addition it showed the longest period of efficiency by 180 days and that is preferable for beekeepers from the economic point of time and cost as mentioned before. Moreover, it proved to be lower in its adverse effect on honeybees which recorded mean number of killed bees every week 6 bees/ colony, and so it is recommended to use this method against varroa mite infestation. This method followed by applied 10.0 gm on topbars/ colony, it recorded 82.847%, the differences between the two methods were insignificant in percent reduction of varroa infestation

within the test period (42 days) of application in colonies under test. In addition the later method of application proved to be longer in efficiency by 160 days and that is also, preferable as previously shown.

6-Effect of Varroa mite control by chemical substances on honeybee colonies. The chemical substances (Mavric) were used against Varroa mite infestation caused marked decrease in life span (percent reduction of longevity) recording 52.42%. On the other hand Varroa infestation caused the highest percent reduction of longevity in newly emerged honeybee workers obtained from untreated control colonies recording 71.75%. The strength of honeybee colonies treated with non chemical substances (formic acid 85%) were stronger than that treated with formic acid recorded 240 inch² sealed brood cells of honeybee workers and 8 combs covered with bees. whereas, the honeybee colonies treated with Mavric, recorded 130 inch² sealed brood cells of honeybee workers and 4 combs covered with bees. On the other hand, untreated control colonies recorded 40 inch² sealed brood cells of workers and 2 combs covered with bees.

III-Differential survival of honeybee colonies infested by Varroa jacobsoni and selecting for natural resistance . Despite the use of chemical and other measures against Varroa mite, very high losses of honeybee colonies have occurred. However, in some untreated apiaries a few colonies were apparently coexisting with the mite. This fact indicates the possibility of breeding for resistance to, or for coexistence with *V. jacobsoni*. Lulincevic (1985). Varroa mite infestation caused loss in honeybee colonies by 50.769% for F1 Carniolan hybrids and 48% for F1 Italian in the first year of selected program (1997-98) and then the losses have decreased to 16.66% for F1 Carniolan and to 15.15.1% for F1 Italian in the second year of selected program (1998-1999). In the third year (1999-2000) were 5.600%, 4.761% for F1 Carniolan and F1 Italian hybrids, respectively. F1 Italian hybrids showed the highest responses to grooming behavior development recording mean percent efficiency of grooming 47.058%. whereas F1 Carniolan hybrids were 24.793. In addition, it is obvious that F1 Italian proved to be the most active in brood rearing activity and then number of bee population. For instance, the total sealed brood area 240 inch², 620 inch² in Spring and Summer (2000) at the end of selected program for F1 Italian and that of F1 Carniolan were 120, 460 inch² respectively. Worker brood in all colonies was lightly infested than that of drones, the mean % infestation in worker brood for F1 Italian (0.4%) was lower than that of F1 Carniolan (0.66%) and so, of drones. In addition, the number of dead mites found were comparatively small ranged 4-10 mites and 5-10 mites for F1 Italian and F1 Carniolan, respectively. It is recommended to use selected program honeybee colonies for tolerant Varroa mite infestation and preferred Italian hybrids of honeybees for its previous reasons and the multiple benefits of selected program.

IV-Effect of using formic acid and oxalic acid against Varroa mite in honeybee colonies on chalk brood infection and bees. The number of missing colonies was high in control (untreated colonies) recorded 6 colonies, while the number of missing colonies treated with oxalic acid concentrations of 4.0% and 3.0% were 3 and 1 colonies respectively. However, there was no dead colonies in treatment with formic acid 85%, formic acid 60% and oxalic acid 2.0% concentrations. The treatment with formic acid 85% concentration has the highest efficiency 100% in controlling varroa mite as well as chalk brood infestations. While, treatments with high concentrations of oxalic acid had a harmful effect on number of live bees in the colony with no useful effect against varroa or chalk brood infestations. In addition formic acid 60% had intermediate effects on controlling chalk brood during control of honeybee colonies infested with varroa mites.

V-Effects of artificial feeding with antibiotic (for control treatments) on honeybee brood diseases. Strict observance of proper management requirements including supply of colonies with good quality food is an efficient measure of controlling Varroa disease. It is known that strong colonies are less affected by the disease than the weak ones. (Smirnov, 1978).

a- Effect of feeding with fresh skim milk, contains Ampicillin and Chloramphenicol (for control treatments) on brood diseases in honeybee colonies. The colonies were fed each 7 days intervals proved to be the lowest percent of missing colonies (16.000%) which 4 colonies were lost from total of 25 colonies infested with Varroa mite compared with control colonies (percent missing of colonies 41.666%) counted 5 colonies from total of 12 colonies were died, which all dead colonies could not overwintering during 1998. On the other hand, the honeybee colonies were fed each 14 days showed the highest percent of missing colonies (23.750%) compared with the mentioned two feeding methods, and it recorded 19 colonies from total of 80 colonies — were died, they could not over

wintering compared with honeybee colonies were fed each 7 and 3 days intervals. The colonies were fed each 7 days proved to be the most honeybee activity and high efficiency in honey production (9.0 kg/ colony) compared with all treated colonies. The honeybee colonies were fed each 14 days showed the highest efficiency in decreasing the percent infestation of chalk brood recorded 0.00% compared to the honeybee colonies under test. The percent infestation of honeybee colonies infested with Varroa mite, Chalk brood and Sac brood before the treatments were ranged 16% - 20% with average 18%, 8.75% - 20.000% with average 11.842% and 15% - 32% with average 21.052%, for the three mentioned diseases, respectively — during Autumn and Winter 1998. whereas the percent infestation of honeybee colonies infested with Varroa mite, Chalk brood and Sac brood decreased and were ranged 6% - 10% with average 6.5%, 0.00% - 8.333% with average 2.631% and 3.750% - 16.666% with average 5.263% for Varroa, Chalk brood and Sac brood, respectively in Spring and Summer 1999. The obtained results showed that the honeybee colonies were feeding each 7 days were better in their strength than that of each 14 days and each 3 days intervals. From the previous results, it could be concluded that supply of honeybee colonies with good quality food (Skim milk) and antibiotic Ampicillin with chloramphenicol in this investigations resulted that these colonies had lower diseases incidence and produced much honey. This reduction in brood diseases Varroa mite, Chalk brood and Sac brood could be attributed to the activation of grooming behaviour in honeybees caused by using of grooming behaviour in honeybees caused by using these antibiotics.

b-Effect of feeding honeybee colonies with food contain antibiotic as control for brood diseases incidence The percent infestation as a total mean of honeybee colonies infested with Varroa mite, chalk brood and Sac brood decreased in generally according to the efficiency of different methods of feeding and control in all tested colonies of the five centers recording 3.400%, 18.16% and 0.704%, respectively — during Spring and Summer 2000. In apiary of Talla center, the methods of feeding and control were used in tested honeybee colonies proved to be the highest efficiency in reduction the percent infestation of the mentioned brood diseases and so, the high production of honey yield 8.750 kg/ colony. The percent infestation in these colonies decreased to reach 2.0% Varroa mite, 0.0% Chalk brood and 0.0% Sac brood diseases. The important reasons of the high decrease in percent infestations in these brood diseases due to the activation of antibiotics were used. The honeybee colonies in apiaries of Quisna, Ashmun and Shebin El-Kom centers were intermediate in reduction the percent infestation of mentioned brood diseases and the honey production except the honeybee colonies treated with formic acid 85% and Aromatic botanical extracts in apiary of Quisna with recorded percent infestation of 3%, 0.0% and 0.0% for Varroa mite, Chalk brood and Sac brood diseases, respectively and produced honey 8.0 kg/ colony in Spring and Summer 2000, nearly similar with the honeybee colony of apiary in Talla center.

VI- Incidence of American foulbrood disease in Egypt.

a-Diagnosis and identification of AFB disease in honeybee (*Apis mellifera* L.) colonies found at Menoufia Governorate. The following tests and clinical symptoms on the infected honeybee colonies proved the incidence of *Bacillus* larvae caused AFB in honeybee colonies in Menoufia Governorate apiaries during 1998. Hoist milk test (Hoist, 1946), Baily test (1981) 9 microscopic diagnosis from infected scales and a positive diagnosis based on clinical symptoms of the diseased larvae as: the capping of the diseased cell becomes moist and darkens in color, the cells of the brood comb were a scattered and irregular pattern of capped and uncapped cells, puncturedappings and atropy brown mass and when a pupa dies its tongue generally protrudes from the scale to the center of the cell (This symptom is characteristic only of pupae infected by AFB) in addition, the photograph pictures taken for AFB disease.

b-Detection of American foulbrood disease (*Paenibacillus* larvae found in honey samples taken from 5 Governorate apiaries Egypt during 1998 — 1999. The examination of honey samples which harvested from different localities to obtain the incidence of *Paenibacillus* larvae caused AFB disease proved the incidence of AFB disease in honeybee colonies Menoufia and Duhia Governorate apiaries.

VII-Evaluating of some compounds in controlling AFB Eight compounds were fed to colonies of honeybees infested with American foulbrood. All those treated with Ampicillin, Terramycin and Tetracycline 4 colonies for each became free of visual evidence of the disease, and when chloramphenicol added for each compound in an other treatment, 4 colonies for mixture compound. The results were the colonies

treated became visually free of the disease in less time than those treated with any other compound alone. Streptomycin alone gave poor control and the result improved when chloramphenicol added to it.