
Studies on microbial diseases of honey bee(*apis mellifera*) colonies

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The essential and valuable role of bees depend upon maintaining a healthy population of honeybees; as they forage for nectar and pollen, play a vital role in the environment and in preserving biodiversity by pollinating wild flowers and many agricultural crops. Depending on the previous facts, the present study had been concerned with the microbial diseases that may affect honey bees health. Samples of different microbial diseases had been collected from honeybee colonies from different localities in Sharkia. Six bacterial isolates had been isolated, purified and identified. Three isolates identified as gram positive bacilli, two were gram positive cocci and one gram negative bacilli. Only two of the six isolates were spore forming (*Paenibacillus larvae* larvae and *Paenibacillus alvei*). *P. l. larvae* was found to have the highest colony count followed by *Melissococcus plutonius* count. Twenty one species of fungi had been isolated, purified and identified (nine sp. of *Aspergillus*, four sp. of *Penicillium*, two sp. of *Paecilomyces*, one sp. of *Ascosphaera*, one sp. of *Alternaria*, one sp. of *Fusarium*, one sp. of *Epicoccum*, one sp. of *Botryoderma* and one sp. of *Scapulariopsis*). *Ascosphaera apis* colonies had the highest colony count followed by *Aspergillus flavus*. The study had screened number of control methods for *P. l. larvae* (the pathogen of American foulbrood disease), *M. plutonius* (the pathogen of European foulbrood), *A. apis* (the pathogen of chalk brood disease) and two sp. of *Aspergillus* (the pathogen of stone brood disease). 1. Eleven antibiotics (amoxicillin+ clavulanic acid, bacitracin, chloramphenicol, doxycycline, erythromycin, gentamicin, rifamycin, sulbactam+ ampicillin, tetracycline, trimethoprim+ sulphamethoxazole and neomycin) had been screened for their antimicrobial effect on the five tested pathogens. *P. l. larvae* showed sensitivity to six of the tested antibiotics and the highest activity was for rifamycin which inhibited the growth of *P. l. larvae* with inhibition zone diameter (44mm). *M. plutonius* developed resistance to eight antibiotics of the tested group. Two of these antibiotics had intermediate activities on *M. plutonius*, while rifamycin was the only tested antibiotic developed antibacterial activity on *M. plutonius*. Three of antifungal agents (exoderil, fluconazole and mycostatin) had screened against the tested fungal pathogens. Fluconazole showed weak antifungal activity against *A. apis*, while it didn't show any activity against *A. flavus* and *A. niger*. Mycostatine didn't exhibit any effect on *A. apis*, weak effect on *A. flavus* and high effect on *A. niger*. Exoderil had the highest effect on the three tested fungi (*A. niger*, *A. flavus* and *A. apis*), respectively. 2. The antimicrobial activities of eleven essential oils

(chamomile, cinnamon, clove, eucalyptus, lavender, lemon grass, oregano, peppermint, ricinus, sweet fennel, wheat germ oil) had been screened for their ability against the tested microorganisms. Cinnamon oil exhibited the highest activity on preventing the growth of tested bacteria and fungi. In the same time, the rest of the tested oils didn't show any activity except; clove oil exhibited weak activity against (*P. l. larvae*, *M. plutonius*, *A. flavus* and *A. niger*), lavender oil exhibited weak effect against *M. plutonius* and lemon grass oil developed weak activity on (*P. l. larvae* and *A. apis*).3. The activities of serial extracts of two medicinal plants (cinnamon and thyme) had been screened. Extraction had been developed by serial of five solvents (petroleum ether, ethyl acetate, acetone, chloroform and ethanol 95%), respectively using soxhlet apparatus. Cinnamon petroleum ether extract was the effective among the tested extracts; exhibited highest antimicrobial activities against microorganisms under test, except ethyl acetate extract of the same plant exhibited the same inhibition zone with *A. flavus*. While thyme extracts exhibited antibacterial activity against *P. l. larvae*.4. The antagonistic activity of two *Trichoderma* species (*T. harzianum* and *T. virid*) had been developed against tested pathogens. Both of *Trichoderma* species had the same effect on the *A. apis* growth; they completely inhibited its growth on the petri dishes. While, *T. virid* exhibited no effect on *P. l. larvae* and *M. plutonius* and weak effect on *A. flavus* and *A. niger* than *T. harzianum*. *T. harzianum* developed high inhibitory effect on *P. l. larvae*, *A. niger*, *M. plutonius* and *A. flavus*, respectively according to its inhibitory effect.5. The MIC values of the tested agents had been measured. MIC of rifamycin 0.78 ug /ul for *P. l. larvae* and 500 ug /ul for *M. plutonius*. MICs of exoderil were (15 ug /ul, 500 ug /ul and 500 ug /ul) for *A. apis*, *A. flavus* and *A. niger*, respectively. MIC values of cinnamon oil were measured as 0.31 mg/ml for *P. l. larvae*, 125mg/ml for *M. plutonius*, 3.1mg/ml for *A. apis*, 12.5 mg/ml for *A. flavus* and 3.1 mg/ml for *A. niger*. Cinnamon petroleum ether extract MIC values for tested pathogens were 200mg, 350mg, 75mg, 100mg and 100mg for *P. l. larvae*, *M. plutonius*, *A. apis*, *A. flavus* and *A. niger*, respectively. *T. harzianum* MIC for tested pathogens were developed. All of the tested *T. harzianum* concentrations against *A. apis* were effective MIC was developed to be ($\leq 19.04 \times 10^3$ spore/ml). MIC for *P. l. larvae* (458.7×10^3 spore/ml) and for *M. plutonius* was ($\geq 458.7 \times 10^3$ spore/ml). *T. harzianum* MIC for *A. niger* and *A. flavus* was the same (38.08×10^3 spore/ml).6. *T. harzianum* activity as antagonism for *A. apis* (chalk brood disease) was developed in the apiary. Four concentrations of *T. harzianum* were tested (38.08×10^3 , 19.04×10^3 , 9.52×10^3 and 4.5×10^3 spores/ml). Three replicates were prepared for each concentration and another three as control. Suspensions of *T. harzianum* were prepared from young cultures (4days) were added to the sugar syrups with adding a suspension of *A. apis*, to confirm that the reason of disease recover would assume to be the effect of *T. harzianum*, not hygienic behavior of honeybee workers. All *T. harzianum* concentrations were effective in preventing the chalk brood disease symptoms to appear in tested colonies.