MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

A PRELIMINARY PILOT STUDY OF SOME BOTANICAL EXTRACTS EFFECT ON NOSEMA DISEASE OF HONEY BEES

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Received:2/4/2022
Accepted: 21/4/2022

Abstract: Nosema is a serious disease that strongly affects bees performance and life. This disease is widespread almost all the world and is highly dangerous fungal infection for honey bees, which occurs with infection of honey bees ventricular epithelial cells by *Nosema* spp. pathogen. This preliminary study is concerned with the bioassay of two chemical drugs and seven medicinal plant extracts against nosema honey bee disease. The laboratory experiment included the effect of these substances on the honey bee workers death and *Nosema* spore count. Lemon peels, lemon grass, wormwood and thyme were more potent as antifungal activity against nosema. Septazole and baycox 2.5% chemical drugs as well as the clove, anise and basil extracts exhibited the lowest activity.

keywords: honey bees, nosema, botanical extracts

1.Introduction

Honey bees are so fantastic organisms because of their social life and astonished-benefit products. They are well organized eusocial super-organisms that live together in their hive. The importance of honey bees is due to their precious products that are all have much nutritional, therapeutic and cosmetic importance (41) and (33). Honey bees are so important pollinators, responsible for cross-pollination of more than 80% of our essential crops. Their great success is owed to their specific products as essential chemical weapons against pathogens and enemies (45).

Honey bees health is a major current concern because of their severe mortality all over the world in recent years (44). This phenomenon is known as colony collapse disorder (CCD), which may happen because of pollution, insecticides and diseases. Infectious diseases are important factors affecting the development and consistency of honey bee colonies (43) and (23). Honey bee diseases impact may vary from minor stress to death, so beekeepers should be aware of the symptoms and management. Nosema disease is a dangerous fungal disease that infects adult honey bees and reduces its life span leading to a decline in bee population mainly after winter season (38) and (39). The causative agent of nosema disease in European honey bees (*Apis mellifera*) was the pathogen *Nosema apis*. Then, the pathogen *Nosema cerana* that infect the Asian honey bee (*Apis cerana*) started to infect the European honey bees (11). The two types of *Nosema* can be differentiated microscopically (12) and genetically (25) and (29).

If colonies are already infested by nosema disease, control methods should be applied. The only effective product for *Nosema apis* control is the antibiotic fumagillin but unfortunately, it kills vegetative forms only (46). There is also a fact that fumagillin is very stable in honey and this is a big problem to honey market (Assil and Sporns, 1991). Also European countries prevent the use of antibiotic fumagillin for treatment of honey bee diseases. Control of honey bee diseases is done by preventive methods in these countries (34). For *Nosema cerana*, fumagillin was not efficient in as a control drug, so

searching for new methods is being done (27). Several troubles may be related to the extended use of antibiotics. Not surprisingly, antibiotic resistant characteristic have evolved (28). However, the use of antibiotics risks persistence of chemical residues in honey, contaminating and diminishing its quality for human consumption. Furthermore, the use of antibiotics may reduce the lifespan of honey bees (3) and (32). Residues in honey are not allowed according to European legislation, but treatments with antibiotics are effected in many other countries (36) and (7).

The need for new and safe sources of the efficient treatment becomes very important. Plants are the great mine, which contains enormous numbers of effective medicinal compounds. Many botanical extracts were tested against honey bee diseases worldwide, especially against nosema disease (13). Several plant extracts showed antibacterial activity against American foulbrood disease (37) and (14). Also, essential oils (22) and propolis have been tested to control American foulbrood. Lastly, the antifungal activity of natural extracts has been reported to inhibit the growth of *Ascosphaera apis* (9). The aim of this study is to screen several plant extracts by testing their efficacy in comparison with chemical drugs for control nosema disease caused by *Nosema ceranae* in honey bees, *Apis mellifera*. The study extended to the effect of the disease and these tested therapeutic materials on the essential organs of the honey bee workers as the midgut and hypopharyngeal gland.

2. Materials and methods

1. Apiary and bees

The study started in a private outdoor apiary located at Meet Fares village, Bani Ebeid district, Dakahlia province (31°04'50.4"N & 31°35'51.8"E). The study was conducted during spring and early summer as clover nectar flow period and nosema disease prevalence, which is being pandemic. The apiary was surrounded with tall walls and covered with a ceiling from reed grass for protection from winds during winter and early spring. The flora found in the apiary area comprised *Eucalyptus* sp., *Poplus* sp., citrus, palms, beans, flax and clover. The tested honeybee race was the local Carniolan of *Apis mellifera carnica* hybrid (F1). The hives were one-chambered 7-frames typical Langstroth type. Every five days, all hives received sugar syrup (1 : 1, w/v) and pollen substitute patties every 5 days for general enhancement of the honey bee colonies development. The hives were inspected for nosema infection for marking healthy and infected colonies. The general known symptoms of nosemosis infection possibility is diarrhea inside and outside the hive walls. One chosen infected colony will be the source of the *Nosema* sp. spores for the artificial infection during the laboratory experiments. On the other hand, a chosen healthy colony will be the source of healthy honey bee workers for bioassay and evaluation of the essential oils of the chosen medicinal plants and herbs as well as the chemical drugs. Both healthy and naturally *Nosema*-infected colonies should be the only continuous sources throughout the study, avoiding genetic variations as well as they never received any treatments except feeding.

2. Tools and feeding:

Clear rounded plastic containers measuring 12 cm in diameter × 10 cm in depth were used after slight handling. Every container was punctured with a hot stainless steel dissecting needle, making 20 pores (2 mm diameter) for ventilation of the caged honey bee workers. Also, another 2 large round holes were made with a hot cutter in the bottom and near the cover of the plastic container. The bottom hole is appropriate for insertion of 5 ml plastic syringe, whose needle was removed and its tip was totally eliminated. The syringe was vertically hanged so that its opening was directed downward, facilitating the honey bee workers feeding with sugar syrup. The other hole is suitable for insertion of Eppendorf tube that was perforated longitudinally with about 2 × 1 cm rectangular hole. It can contain about 2 g of the clover pollen patty for honey bee workers feeding. Each container was provided with a small piece of wax fixed to its inner side for ease of movement and providing familiar environment simulating the hive.

All hives were received sugar syrup and pollen patties for enhancement of the colonies development before the study procedures. The honey bee workers were fed the same syrup and

patties with the same ratios throughout the laboratory experiments. For apiary regular feeding, fresh sugar syrup was prepared by dissolving white sugar cane in water with an equal ratio (1 : 1, w/v). In the all apiary, each hive was received 500 ml of sugar syrup every week. For the laboratory experiments, the syrup was prepared using distilled water with the same equal ratio by means of measuring flask. Each experimental group was received 5 ml sugar syrup in the syringe according to the previous handled equipment. The pollen supplement was represented by clover pollen as a natural source of honey bees protein feeding. Pollen was collected by the beekeeper using the pollen traps from the apiary during the previous spring as the nectar and pollen flow season. Pollen was directly kept at -20°C for about 8 months. Such low temperature degree is the most suitable to preserve the main precious constituents of pollen without a great decline of its nutritional value. Pollen patties were prepared by blending the clover pollen with pure strained clover honey with a ratio of (1 honey : 10 clover pollen). The patty should be mixed very well to become completely homogenous and the patties were freshly prepared in the same day of feeding. The same patties were used in feeding of honey bees in the apiary or in the laboratory experiments. Each hive was fed 500 g and each experimental group was received about 2 g and they were continuously replaced after 2 days or just after complete consumption by honey bees.

3. Experimental infection

A sample of 20 honey bee workers was picked up from the marked naturally *Nosema*-infected colony and another sample of 20 workers from the healthy colony. The workers were transported to the laboratory and dissected under a streomicroscope, and then their midguts were removed. The infected alimentary canals have amber colour instead of the normal white and have no ventricular constrictions. Then, the 20 infected ventriculi were placed in 20 ml distilled water, manually grinded with a mortar. A few drops of the suspension were mounted on a glass slide, covered with glass cover slip. Finally, the slide was examined by means of a compound Leitz microscope with up to 400× magnification to ensure the presence or absence of the infection with *Nosema* spores in the colonies. The same was applied for the healthy colony to ensure the absence of infection. A sample of 100 honey bee workers was collected from the aforementioned naturally *Nosema*-infected colony and transported to the laboratory. The infection was determined as previously shown and the whole infected honey bee workers were manually smashed in a mortar containing 100 ml distilled water. The resulting suspension with the debris was filtered by a glass funnel containing a small clean piece of gauze. A few drops from the filtrate were examined by a compound Leitz microscope with up to 400× magnification. The number of *Nosema* spores was counted by using haemocytometer slide (Neubauer grid) and the spore count should be sufficient for the honey bee workers infection (not less than 10^4, if was it is considered not detected ND) (10). The fresh infection solution was with sugar syrup was prepared by centrifugation and addition of precipitate of *Nosema* spores to 50% sucrose solution to obtain a final concentration of about 20×10^6 spores/ml (20).

4. Treatments of chemical drugs

Two chemical drugs were tested in this work against nosemosis disease. The first was the antibacterial therapeutic drug commercially called septazole and purchased from Alexandria Co. for Pharmaceuticals & Chemical Industries, Alexandria, Egypt. This drug comprises two effective chemical substances; the main one is sulfamethoxazole with a concentration of 40 mg/ml and the second is trimethoprim with a concentration of 8 mg/ml. The second drug tested against nosemosis infection was toltrazuril. It is a veterinary antibiotic used against some poultry, sheep and cattle protozoans and coccidians. The commercial trade name is (Baycox 2.5%) as an oral suspension. It was purchased from International Free Trade Corporation (IFT), Mokattam, Cairo on behalf of Baver, Germany.

5. Treatments of botanical extracts

Seven plants and herbs were tested against nosemosis. Certain portions of these plants and herbs were chosen according to its common use in herbal medicine. The lemon peels, clove dry fruit and anise seeds were purchased from the markets; while, the leaves of basil, thyme, lemongrass and wormwood were collected

from the farm of Faculty of Agriculture, Mansoura University, Mansoura, Dakahlia Governorate, Egypt.

The collected plants and herbs were cleaned from debris and washed with tap water. The fresh and vegetative parts were cut with a mixer to small pieces. The clove bud and aniseeds were grinded with a grinder to fine particles. Each plant material was hydrodistilled for essential oils extraction. The flask containing the plant was provided with sufficient distilled water and placed above the heater until boiling the mixture. The elevating vapours produced from the flask containing the plants were passed into the condenser and condensed (cold water circulation) into the receiving flask for 8h. After that, the essential oil was extracted from the immiscible oil-water mixture with methylene chloride solvent and dried over anhydrous sodium sulphate for complete dehydration, filtered and then evaporated in the room temperature. The residual essential oils were transferred to dark glass bottles and tightly closed with plastic stoppers and kept in the deep freezer at -14°C until use (15).

6. Experimental design

All experimental containers received 250, 500 and 1000 ppm of its own treatment except the +ve control one.

- Container (1): was experimentally infected and untreated (+ve control)
- Containers (2-4): were artificially infected and treated with septazole drug (active substances are sulfamethoxazole and trimethoprim), respectively.
- Containers (5-7): were artificially infected and treated with Baycox 2.5% (toltrazuril).
- Containers (8-10): were artificially infected and treated with lemon grass essential oil, respectively.
- Containers (11-13): were artificially infected and treated with thyme essential oil, respectively.
- Containers (14-16): were artificially infected and treated with wormwood essential oil, respectively.
- Containers (17-19): were artificially infected and treated with clove essential oil, respectively.
- Containers (20-22): were artificially infected and treated with anise essential oil, respectively.
- Containers (23-25): were artificially infected and treated with basil essential oil, respectively.
- Containers (26-28): were artificially infected and treated with lemon peels essential oil, respectively.

7. Experimental procedure

In the healthy colony, after 20 days from the beginning of egg-laying, the brood became capped in the frames just before adult workers emergence. It was removed from the hive and transferred to a tightly closed frame carrier that was covered with a wire mesh that prevents workers escape. Then, it was transferred to the laboratory and kept in an incubator at 34°C and 60% relative humidity. After emergence of the baby worker bees, they were considered zero day old according the date of emergence. They were used in the subsequent bioassay experiments. After 24h of adult honey bee workers emergence in the laboratory incubator, the experiment was started. This is considered the 1st day according to the previously-mentioned design of the preliminary experiment. Each experimental group consists of the aforementioned handled clear rounded plastic container that was packed with 50 newly emerged adult honey bee workers. Each group was fed 5 ml freshly prepared and healthy sugar syrup (1:1, w/v) with the plastic syringe and 2 g pollen patty in the Eppendorf tube. Every two days, all plastic syringes were removed from the containers and their holes were covered with adhesive papers, preventing honey bee workers escape. Then, they were rinsed with running tap water and finally with distilled water and refilled with freshly prepared healthy syrup with the same amount. Also, the Eppendorff tubes were replaced with new ones packed with the freshly prepared patty with the same amount.

In the 1st day, all plastic containers were incubated at 33°C and 60% relative humidity and decreased to 31°C and 30°C on the 2nd and

3rd day, respectively. By the 3rd day post-emergence, all groups were infected with *Nosema* inoculum according to (20). The syringes were removed and their holes were covered for 2 h before application of the infection syrup to assure feeding. They were rinsed and refilled with the aforementioned freshly prepared artificial infection sugar syrup (1 : 1) and introduced to the honey bee workers for 24 h to achieve infection with *Nosema* sp. spores. On the 4th day, all feeding materials were removed and replaced with healthy freshly prepared sugar syrup and new pollen patties as previously done. On the 10th day, the 2 chemical drugs and 7 essential oil treatments were introduced to the different groups with the aforementioned concentrations according to the experimental design. All treatments of chemical drugs and essential oils were applied to the worker bees in the sugar syrup with a ratio of (1 : 1) except in case of the healthy and artificially infected groups. This step of the preliminary experiment was implemented in 24 h. On the 11th day, the feeding sugar syrup and patties were replaced with freshly prepared, healthy and untreated amounts. On the 20th day, the number of dead honeybee workers in each plastic container were counted and recorded. It was randomly picked up a sample of 10 individuals from each container, placed in a mortar containing 10 ml distilled water and manually squashed. Each sample suspension was manipulated as previously mentioned, examined by using a haemocytometer slide and *Nosema* spores were counted and recorded.

3. Results and Discussion

This experiment was preliminarily carried out as a survey of some chemical drugs and essential oils that have antimicrobial properties against nosemosis. The numbers of the dead workers during the time course of the preliminary experiment were recorded. Also, the spore count of *Nosema* was recorded per each honey bee worker. In case of *Nosema*-infected group, a positive control group, the numbers of dead workers was 39 with a death percentage of 78%. The spore count of *Nosema* was 20.5 million spores in the dead sampled honey bee workers. In case of the chemical drug septazole-treated group, the numbers of dead workers were 18, 19 and 22 with death percentages of 36, 38 and 44%. While, the spore count of *Nosema* was15, 20.25 and 19.25 million spores per each dead worker with drug concentrations of 250, 500 and 1000 ppm, respectively. The other tested drug was baycox 2.5%, which its concentrations resulted in 25, 24 and 23 dead workers with death percentages of 50, 48 and 46%. The spore count of *Nosema* was 11.25, 10.5 and 10 million spores for the three concentrations of 250, 500 and 1000 ppm, respectively.

The findings of lemongrass showed numbers of dead workers of 12, 6 and 6 with percentages of 24, 12 and 12%. The spore count was 5.25, 3 and 3 million spores for the concentration of 250, 500 and 100 ppm, respectively. The wormwood plant resulted in 14, 9 and 8 dead workers with percentages of 28, 18 and 16%, while, the spore count was 5, 4.25 and 3.75 million spores for the three concentrations of 250, 500 and 1000 ppm, respectively. The lemon peels showed numbers of dead workers of 24, 9 and 9 workers with percentages of 48, 18 and 18%, while, the spore count was 6.75, 2.5 and 2 million *Nosema* spores for the three concentrations, respectively. The treatment with thyme resulted in numbers of dead workers of 32, 16 and 15 with percentages of 64, 32 and 30 %. The spore count of *Nosema* was 4.5, 3.25 and 2.75 million spores in case of the three concentrations of 250, 500 and 1000 ppm, respectively. Clove-treated group showed 21, 18 and 20 dead honey bee workers with percentages of 42, 36 and 40%. *Nosema* spore count was 9.5, 8 and 7.5 million spores with the previous three concentrations, respectively. The treatment with anise herb resulted in a number of dead workers of 16, 37 and 35 with percentages of 32, 74 and 70%; while, the spore counts of *Nosema* were 13.5, 17.5 and 16.25 spores with the three concentrations, respectively. Basil-treated group showed that the numbers of dead workers were 43, 40 and 40 with percentages of 86, 80 and 80%. The spore count of *Nosema* was 11.5, 10 and 10.25 million spores in case of the three concentrations of 250, 500 and 100 ppm, respectively.

The previous findings of the tested septazole and baycox 2.5% revealed that these two chemical drugs showed a moderate ameliorative effect against *Nosema* spores, the causative agent of nosemosis in comparison

with the *Nosema*-infected group or the positive-control. This effect of the two standard drugs is approximate between them and between the three used concentrations in case of dead worker numbers. In case of septazole treatment, the spore count was nearly as high as the *Nosema*-infected group and in baycox 2.5%, it was in a moderate range when compared with the infected group. This may be due to the resistance acquired by continuous usage and overdosing of these chemical drugs as antimicrobial treatments in the apiary. The treatment with the medicinal herbal extracts showed that the most potent one was the lemongrass. It achieved the least worker death percentages. The least spore count was recorded in case of thyme- and lemongrass-treated groups. The next rank was for the wormwood and lemon peels herbal extracts with higher dead worker numbers and spore count. In general, the weakest antifungal activity was recorded from the basil-, anise- and clove-treated groups with a ranking from the weakest to the strongest (Tables 1 – 3 and Figs. 1 – 3).

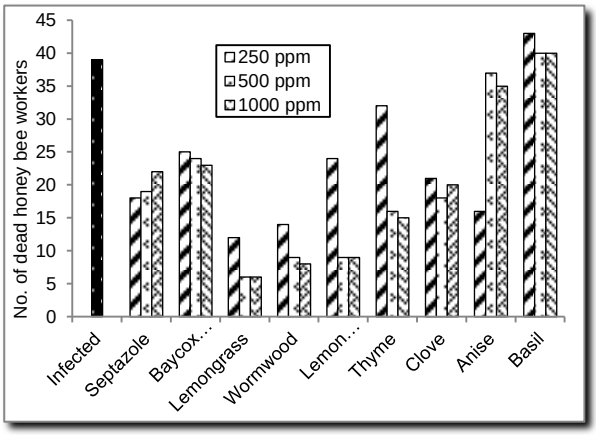

Fig. 1. Numbers of dead workers.

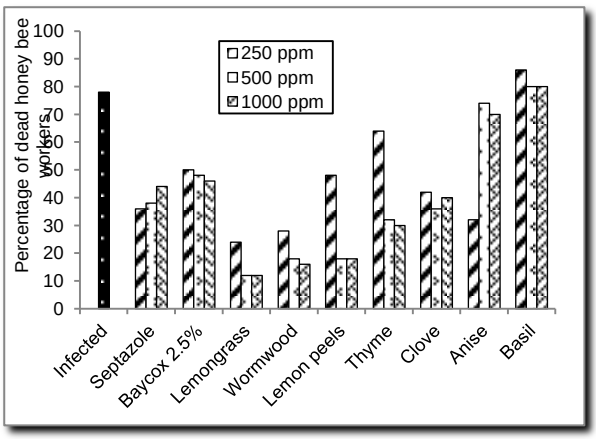

Fig. 2. Percentage of dead workers.

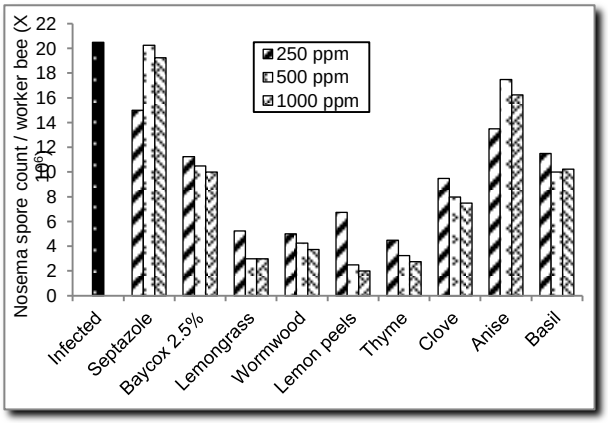

Fig. 3. *Nosema* spp. spore count.

The results showed that trimethoprim-sulfamethoxazole drug was most potent and effective than toltrazuril. Though, toltrazuril increased the percentage of dead workers with 28% when compared with trimethoprim-sulfamethoxazole. This is may be an indication to toxicity of this drug against the honey bees. Fumagillin is the most classic potent antibiotic used long time ago in treatment of nosemosis. Due to the increased resistance of pathogen toward the drug it should be necessary to find new effective chemical and herbal-derived antibiotics. Fumagillin is the only antibiotic approved for control of nosema disease in honey bees and has been extensively used in United States apiculture for more than 50 years for control of *Nosema apis*. It is toxic to mammals and must be applied seasonally and with caution to avoid residues in honey. Fumagillin degrades or is diluted in hives over the foraging season, exposing bees and the microsporidia to declining concentrations of the drug (27). Hence, the need for new drugs and substances control nosema evolved. In agreement with the findings of this work, invasive mycosis has significantly increased in frequency among immunocompromised hosts leading to excessive morbidity and mortality. The combination of sulfamethoxazole (SMX) and trimethoprim (TMP) has been used extensively for the treatment and prophylaxis of infections by various microbes as *Aspergillus fumigatus* and *A. oryzae* (Hida *et al*., 2005). The reduction percentage of honeybee workers in the colonies infected with *Nosema apis* that treated with bee venom were 41.0, 50.0, 50.8, 46.0 and 3.7 % for feeding, spraying for bee venom solution, positive control (Artemisia), (Septazole) and negative control, respectively

(6). It was reported that the constant treatment regimens with toltrazuril against *Cystoisospora suis* (coccicoid) infection in piglets are being applied in the intensive production systems for the last two decades. The possibility of resistance development has not been addressed so far despite limited availability of treatment alternatives. Recently, a pig producer in The Netherlands who routinely used toltrazuril complained about diarrhea in suckling piglets in the absence of bacterial and viral pathogens, and oocysts of *C. suis* could be isolated from feces of affected litters. Treatment with the recommended (20 mg/kg) dose of toltrazuril completely suppressed oocyst shedding and diarrhea in group Wien-20 (42). Also, the tested *Eimeria* isolate was resistant against toltrazuril, and resistance was seen in both pathogenic and non-pathogenic species. In addition, no significant differences in faecal score, growth, gross pathology or histological changes were identified between the two groups. The pathogenic *E. ovinoidalis* was the dominant species, and no significant difference in the individual prevalence of *E. ovinoidalis* post-treatment was found between treated (66.9%) and control lambs (61.9%). Other species identified included *E. parva*, *E. marsica*, *E. faurei*, *E. pallida*, *E. ahsata* and *E. bakuensis* (35).

The most potent substances were lemon peels, lemon grass, wormwood and thyme essential oils. They exhibited the lowest percentage of dead honey bee workers and *Nosema* spore count. This agreed with other researchers as Abou-Shaara (2018) who reported that diluted honey mixed with lemon juice used against *Nosema*. To realize these objectives, diseases of brood and adult honey bees were surveyed over one year. Under field conditions, it reduced the infection by 13.33%. The percentage of survived bees was significantly higher than infected bees without any treatments over the experimental period. Also, (1) tested the antifungal activity of fresh lemon peels against seven pathogenic fungal strains like *Saccharomyces cerivicae* and *Candida albicans*. As a result, the yield of essential oil, methanol, and ethanol extracts were 0.78%; 9.8%; 10.05%, and 0.64%, 8.3%, 8.9% in Marrakech and Kenitra, respectively. The minimum inhibitory concentrations (MIC) were tested at concentrations ranging from (0.1, 0.25, 0.5, 1.25 and 2.5 mg/ml) as wells as the minimum concentrations of antifungals (MFC). Also, zones of inhibition were recorded extend from 9 to 36 mm and from 8 to 18 mm in the concentrations of ethanolic extracts, the zones inhibition ranged from 10 to 26 mm and from 9 to 18 mm in the concentrations of methanolic extracts, and the zones inhibition ranged from 20 to 34 mm and 10 to 20 in the concentrations of essential oil for Marrakech and Kenitra, successively. The minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) to the lower concentration, as opposed to *Saccharomyces cerevisiae*, is 0.1 mg/ml), minimum inhibitory concentrations (MIC) and minimum antifungal, concentrations (MFC) to higher concentrate against candida spp1 are 2.5 mg/ml). The results showed a difference in yields due to the difference in solvents and also in regions. The highest antifungal potentiality was exhibited by the ethanol followed by the methanol followed by the essential oil. Therefore, increased yield offset by high fungal activity, because of the difference in environmental conditions, climate, lack of water, distance, proximity to the sea and elevation, genes, extraction and season. The activities of the extracted oils depend on the availability of the active constituent based on the use of a solvent.

The study of (31) evaluated the efficacy of lemongrass essential oil in the control of the *Aspergillus brasiliensis*. In vitro and serial microdilution tests were carried out at different concentrations of essential oil and citral, which corresponds to 72% of the total oil composition. Inhibition of fungal growth on contaminated wheat grain was evaluated. The in vitro test results showed that the essential oil has fungicidal potential at concentrations from 0.6 µl/ml, the minimum inhibitory concentration was determined at 0.8 µl/ml. The tests with citral showed fungal control at concentrations from 0.6 µl/ml onwards. For wheat grain, fungal growth inhibition was obtained at the concentration of 1.6 µl ml^{-1}. The essential oil of *Cymbopogon flexuosus* showed fungicidal activity against the fungus *Aspergillus brasiliensis*. In the same concurrent, the findings of anti-nosemosis activity of wormwood aqueous extract agreed

with those of (30). It was evaluated the anti-nosemosis activity of aqueous, ethyl acetate (EA), and butanol (BuOH) extracts of *Artemisia dubia* and *Aster scaber*. It was performed both in vitro and in vivo toxicity for all the extracts and also carried out anti-nosemosis experiments. The aqueous extracts showed more potent anti-nosemosis activity than the EA and BuOH extracts. The antifungal activity of Artemisia was assured by the study of (26). The results of the conducted studies indicate that Artemisia herb extracts are able to reduce the growth of microorganisms recommended by the WHO. The investigated extracts showed the potent bacteriostatic action against the cocci or rod-shaped microflora. The antimicrobial activity of Artemisia L. herb extracts directly depends on the concentration of ethanol as the solvent. Artemisia vulgaris herb extracts (solvents – 70% and 90% ethanol) and *Artemisia abrotanum* herb extract (solvent – 90% ethanol) show synergism of antimicrobial activity with erythromycin in relation to *Staphylococcus aureus* with efflux mechanism of MLS-resistance. The obtained Artemisia herb extracts can be used to create antifungal drugs, as well as antimicrobial drugs (against gram-positive and gram-negative bacteria).

In a Turkish study, (47) investigated the effectiveness levels of essential etheric oils against nosema infection. 21 colonies obtained from Ordu Region, equalized physiologically and divided into 7 groups. Nettle, laurel, eucalyptus, thyme, garlic oils (after dilution of 0.48%), and apple cider vinegar were given to each colony 6 times at 3 days intervals of 3 ml/L to 1:1 ratio syrup (prepared for each separate group). Adult bee samples were taken from all colonies before each additional feeding, and *Nosema* spore counts were performed. The results of thyme and clove agreed with some workers. The antimicrobial activity of thyme depends on their chemical constituents especially thyme essential oil. demonstrated the effectiveness of thyme essential oil against the food-related bacteria and fungus. The antimicrobial potential of thyme essential oil is related to its contents of phenolic compounds (thymol) and terpene hydrocarbons (γ-terpinene), respectively (40). A third main agent in thyme according to its fraction is p-Cymene displays synergistic antibacterial action in combination with γ-terpinene and thymol (18); (21). In the same way, three essential oils were selected: clove, lemongrass and thyme. They were screened for antifungal activity against *Eurotium* spp. with different methods: micro- and macro-dilution and agar-diffusion. Growth/no-growth data were used to develop models for all three methods. Clove exerted the strongest antifungal activity with an inhibitory concentration of 0.075%, 0.035% and 0.05% through respectively micro-dilution, macro-dilution and agar diffusion. For thyme the following values were obtained: 0.775%, 0.070% and 0.100%. This means that the antifungal activity of thyme is 10 times lower in micro-dilution and 2 times lower in macro-dilution and agar diffusion compared to clove. Through micro-dilution, lemongrass was found to have the second highest antifungal activity (0.25%). When used in the volatile atmosphere of dried apricots and in macro-dilution, the antifungal activity of lemongrass was the lowest, with respective values of > 0.2% and 0.105% for G/NG prediction (17).

(16) showed that feeding honey bee on Anise honey by concentration 150 g/colony have the lowest infection percentage 28.78% then Anise 100 g/colony 32.94% and Anise 50 g/colony 37.89% compared with control 65.11%. In the second place Fennel honey general mean 43.11%. Finally, Marjoram honey general mean 62.76%. On the other hand, the effect of these aromatic honey samples back to that antioxidant activity of the three types of honeys represented in flavonoids value. The chemical analysis of honey samples was carried out to clarify the beneficial compounds that have an effect on nosema disease. Results showed that the highest flavonoids value was found in Anise honey (14.02) followed by Fennel honey (9.11) and finally Marjoram honey (8.24). The results of basil agreed with those of (19) who use Protofil for the control of nosemosis. Protofil is a maceration of several plants as chamomile, marigold, mouse tail, thyme, basil, mint, sea buckthorn, rosehip, plantain, wormwood, acacia flowers, linden) in alcohol at 70°C.They were tested together with Protofil and sunflower honey on honey bees artificially infected with *N. ceranae*. However,

no treatment resulted in 100% negative PCR results. Furthermore, using cage assays, infected bees do not have the opportunity to get rid of the infective spores (by defaecation) even if they are potentially inactivated by the bioactive medicinal additive. It could be concluded that the lemon peels, lemon grass, wormwood, thyme and septazole drug were the most potent substances. The essential oils of the tested medicinal plants need further studies to identify the most potent one and their effective constituents for control of nosema disease.

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