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Evaluation of the Efficiency of the Aqueous and Alcoholic Extract of Propolis in Reducing the Incidence of *Nosema Cerana* Disease on *Apis Mellifera* Honey Bees

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Abstract. The results of the study showed the superiority of the alcoholic extract of propolis in reducing the infection of Nosema on honey bees to the lowest levels, as it reduced the number of spores to the lowest possible number, especially when using treatment with protein feeding, with an average of 214.20 spores / bee, which positively reflected on the physiological and vital characteristics of the bee's body (age of the bee). Rectal acidity, moisture content of the bee's body and fat content of the bee's body), these characteristics are considered as evidence of the health of the bee's body, with averages of (50.10 days, PH 4.95, 64.52%, and 81.60%), respectively, which did not significantly differ from the percentages of most of the above traits. The male of the extract, when adding the treatment with sugary nutrition followed in reducing the infection rate when using the treatment with the aqueous extract achieved good and satisfactory results, while the infection was high and the coefficients values were low in the comparison treatment, and the highest average number of spores was 2988.70 spores / bee, which was negatively reflected on the physiological characteristics, vitality, activity and health of honey bees, which amounted to (21.10 days, PH 5.85, 89.74%, and 5.95%), respectively.

Keywords: Propolis , alcoholic extract , Aqueous extract , *Nosema cerana* , honey bee ,protein dough.

Introduction

Honey bees face a very great threat in many countries of the world, including Iraq (Abd Al-Hameed and Hadi, 2020, Al-Sayegh and others, 2018), where honey bees in their various stages, both incomplete and adult stages, are exposed to many diseases that can cause the elimination of most of the colonies present in the apiary in a short period or on the entire apiary. It may furthermore spread to neighboring apiaries with the progression and spread of infection, causing great losses, whether at the individual or the state level (Ansari and AL-Ghamdi, 2017). Globalization has provided opportunities for parasites and pathogens to cross borders geography and the expansion of acquiring new hosts, as fungal diseases are among the most common diseases affecting adult bees and mostly widelyspread in all parts of the world as they work to eliminate many colonies, which cause a decrease in the rate of honey production and cult members (Chen et al., 2010). Years ago the bees began to suffer from health problems, and perhaps the most serious of them was the exposure to a group of dangerous disease pests that have caused losses to millions of colonies worldwide (Fries et al., 2013), the most important of which is the Nosema disease or the so-called Nosemosis disease, which causes great economic losses. For example, the percentage of losses of honey bee colonies in the United States of America reached 29% and 32% over the past years (Van Engelsdorp et al., 2010) and in Europe the average loss of honey bee colonies reached 20%. It is believed that hunger and parasites are the main causes contributing to this phenomenon (Coloss, 2009), while in parts of Asia it reached 20% During the winter of 2008-2009, about 10% of the total 2.3 million honey bee colonies in the United States died, and the phenomenon of disappearance and deterioration of bees, or what is known as CCD (Colony Collapse Disorder) was associated with Nosema disease, as this phenomenon was recorded for the first time in honey bee hives in America In the northern year 2006 (Van Engelsdorp et al., 2007), the global bee, Apis mellifera, is infected with two types of unicellular spores Microsporidia belonging to the genus Nosema, namely; Nosema apis and Noseam cerana, and they are considered highly specialized fungi belonging to the genus Microsporea Fries (2010), as they infect cells epithelial cells in the

intestinal wall and lead to disorders in the digestive system in adult bees, which negatively affect colony productivity and greatly reduce the average age of worker bees and thus their survival (Botias, 2013 and Huang, 2012) besides, cause the collapse of colonies and their migration (Fries and Paxton, 2013) and as a result of the effect on the growth of fatty bodies and the reduction of levels of proteins and amino acids in hemolymph of bees (Gajger et al., 2009) and since the description of Nosema apis disease at the beginning of the last century by the scientist Zander), (1909), Nosema infection is transmitted horizontally among members of the sect by By ingestion of spores from contaminated food, or through contaminated water, or through the exchange of food between members of the bees in the process of Trophallaxis, and this step crosses a path of transmission from one bee to another, or infected imported queens and their companions, worker bees, which are considered a source of infection, as well as during their movement from one area to another (Giersche, 2009 Pasca; Contaminated flowers and pollen are considered a source of infection for any sensitive host that comes into contact with those flowers and works to disperse Nosema spores and transfer them to new hosts (Graystock et al., 2015), as well as sexual intercourse and cleaning contaminated cells is a source of infection. It has recently been recorded that Nosema spores were transmitted to by air (Sulborska et al., 2019), and in order to control the infection and prevent its spread, several preventive operations must be carried out, including disinfection of equipment, replacement of old tires, exposure of cells to the sun to reduce humidity and the use of natural extracts that work to enhance and strengthen the immune system, the most important of which are wormwood and thyme And mint, dracaena, star anise, cinnamon, turmeric, propolis, resveratrol, prebiotic compounds, probiotics and other natural substances, and giving therapeutic doses to avoid the spread of the disease. Chemotherapy is often recommended in the fall and spring, especially in cases of severe infection. The fungus Aspergillus fumigatus has been tested and shown to be effective as a treatment for Nosema apis disease on the honey bee A. mellifera (Borges et al., 2021). And Borges.(2015) showed that the use of food supplements that contain prebiotics and probiotics may help control Nosema diseases, especially Nosema cerana that affects honey bees, and that acacia gum is the most effective type in reducing the severity of the disease despite the high mortality rate as a side effect and that Protexinc concentrated single chains of Protexinc1 are able to reduce these spores and increase bee survival. Due to the role that microbiota in the small intestine play in bee health and immunity, supplements containing prebiotics and probiotics may help control N. ceranae, use Suwannapong et al. (2018) and Mura et al. (2020) propolis extract in bee feeding in cages with different concentrations (0%, 50%, and 75%). The result was that the treated bees had higher hemolymph trehalose levels, and the protein level in the subpharyngeal glands was similar to its levels in uninfected bees. Accordingly, treatment with propolis extract can be considered as a promising alternative to fumagelin and other chemicals, and is applicable for improving the health of honey bees.

In view of the lack of in-depth research studies on Nosema disease in Iraq and to find alternatives to chemicals, this study was proposed, which aims to:

1- Evaluation of the efficiency of the aqueous and alcoholic extract of Propolis in reducing the infection of Nosema cerana on the honey bee Apis mellifera
2- Reducing the risks of chemical contamination of honey due to the use of chemical pesticides in the treatment of bees and relying on natural materials such as propolis produced by bees, which have proven their efficiency and effectiveness against diseases that affect honey bees.

Studying the effect and effectiveness of the natural compounds of propolis on some vital and physiological characteristics of honey bees, which included (estimating the number of spores in the studied samples, estimating the age of the worker bee, estimating the pH in the rectum, estimating the fat percentage and estimating the moisture content).

Materials and Methods

study was conducted during the academic year 2022-2023 in the insect and apiary laboratory of the Plant Protection Department / College of Agriculture and Forestry / University of Mosul. The study included the following:

1- Studying the effect of the aqueous and alcoholic extract of propolis in reducing the infection of Nosema cerana disease on the honey bee Apis mellifera.

Preparing and preparing the extract

To obtain the alcoholic and aqueous extract of the propolis, the raw propolis was placed in the home refrigerator (frozen) for 24 hours, then the propolis was ground by an electric grinder and became a powder form. To prepare the alcoholic extract, 100 g of the raw propolis was dissolved in 100 ml of 70% ethanol. The filtrate was placed in a

glass vial and left at 31 °C in the dark for 24 hours, then the extract was filtered by filter paper in order to get rid of wax and other impurities. A concentration of 50% of this extract was prepared in distilled water (volume / volume) for the purpose of conducting the study . As for the aqueous extract, it was prepared by dissolving 100 g of the powder in 100 ml of boiling water and left for 24 hours, then it was filtered and filtered from impurities, and a concentration of 50% of this extract was prepared in distilled water (volume / volume) for the purpose of conducting the study.

After preparing the extracts and the required concentration in the study, a factorial experiment was carried out according to a complete random design, which included two types of alcoholic and aqueous propolis extract, which were presented with feeding to bees, in two ways, viz. the first with sugar solution, and the second with protein paste. As for the comparison treatment, it was left without any addition of materials. treatment, with 5 replications for each treatment (one cage for each replicate).

Executing the experiment

The experiment was conducted inside cages for the purpose of conducting an industrial infection on the bees confined inside them in order to study some of the vital and physiological characteristics of worker honey bees. Nosema spores to the trapped worker bees, where the bees were examined again after the occurrence of infection to ensure the presence of infection:

The experiment was conducted in a dark room similar to the cell conditions, at a temperature of 26-30 °C and relative humidity (70-75%) so that the conditions were similar or close to spring conditions, where (30) cages were manufactured in the local market, each measuring 20 x 20 x 20 cm in front of it has a glass front facing it with a cover of thin wood for the two side facades and the base. As for the upper cover, it was made of thin wood that can be opened and closed, through which water and therapeutic nutrition were provided. The cages were equipped from the front side of the glass cover with a piece of wax with hexagonal eyes occupied for the bees to rest on. And storing the food item inside these eyes and then consuming it, young workers at the age of one day were collected from the maternal cells at the rate of 50 workers per cage and transferred to the inside of the cages, after ensuring that the samples are free of Nosema spores by microscopic examination. For the number of germs in the resulting suspension, amounting to 400 spores, by feeding the bees trapped inside the cage with a sugar solution at a concentration of 1:1 contaminated with Nosema spores (sugar solution + Nosema spores), the samples were examined after 24 hours to ensure that infection occurred, and the examination showed that no infection occurred. The examination was conducted 48 hours after the industrial infection was carried out, and the examination did not show that the infection had occurred. Three days after the infection was conducted, the examination was re-examined and it was found that the infection had occurred with Nosema disease, as the readings were taken until the death of all the bees. The experiment was designed according to the CRD random design. It included three treatments with five replicates for each treatment as well as comparison treatment and the studied characteristics are:

1- Estimating the number of spores in the samples

Microscopic examination method:

In order to be accurate in calculating the number of spores in each sample, the Haemocytometer method was used to estimate the number, where 50 representative workers were taken for each treatment, the samples were taken randomly as observations for statistical analysis, and the method of work was adopted by Hijazi (1997) and Al-Sayegh et al. (2018).) which is next:

- I took a drop of the spores suspension that had been previously prepared by means of a needle with a loop.

Putting the content of the node in the counting chamber, and when the node and the spores it contains touch the surface of the counting chamber, the spores suspension slides into the counting chamber to fill it with a volume of 1 ml.

- It must be ensured that there are no bubbles under the slide cover after placing it.
- I left the slide until the slide settles before the counting process.
- The slide was moved under the microscope lens and the measurement area was determined after adjusting the lens until the spores appeared clearly.
- Five squares distributed in the center of the slide and the edges defined by double lines were selected, each square containing 16 small squares to calculate the number of spores present in (80) squares of the smaller squares to complete the counting process.
- According to the number of spores present in the center of each square that touched the upper and left double line, and neglected the spores that touched the lower and right double line, the count was carried out in a regular way.
- If the spores are not uniformly distributed, i.e. accumulated somewhere in the counting room, the preparation is repeated again after re-mixing the sample to homogenize the sample.

- The dimensions of each of the least squares in the counting room = 0.05 x 0.05 x 0.1 mm3
- So the total volume = 0.0025 mm3 and this = 1/4000 of 1 mm.
- The following equation was adopted to calculate the number of spores in each bee:

Number of spores / ml = Number of spores / 80 x 4 x 10 6

2. Estimation of longevity of worker bees and percentage of bee death in cages

The average lifespan of the worker bees for bees kept in cages was calculated after the death of all workers in the experimental cages, and the averages were extracted on the basis of the longevity of each worker. Al-Sayegh (1988).

2- Determination of rectal pH

For the purpose of preserving the contents of the rectum from loss and the inability to extract the rectum only to estimate the pH, because we need a large number of worker bees, the pH was estimated in the whole abdomen, where the stomachs of five workers were cut out of the workers who were weighed while they were alive without using any chemical substance in the killing process was transferred to a ceramic mortar, and the contents of each refining of each treatment were separately grinded until the contents were homogenized with the addition of a quantity of distilled water so that its final volume became 20 cm3, and for the purpose of reading the pH, the pH device was set up and the temperature inside. The laboratory was equipped with the device, and the device was set using two types of buffer solutions, which are PH 7 and PH 9, then the readings were taken after adjusting the device for the parameters to be read according to the method of Owais (2018) and Al-Sayegh (1988).

4- Determination of moisture content

Five Samples were taken from the worker bees per week, and then weighed while they were wet with a sensitive balance of the type Scaltec, then the samples were dried in an electric oven of the type (Memert) at a temperature of 70 ° for 24 hours, and the weight was taken after drying with the same scale, and the percentage of moisture, which is called the fluid coefficient, was calculated. According to the method of Abdul (1981) and

$$\text{The percentage of moisture in the body} = \frac{\text{Wet weight of bees - Dry weight of bees}}{\text{Dry weight of bees}} \times 100$$

5- Estimation of fat content in bees

The percentage of fat in the body of bees fed with an aqueous and alcoholic extract of propolis was estimated according to the method (Less (1970) using the Soxhlet apparatus based on Al-Sayegh (1988) using the following equation:

$$\%\ \text{of fat in the sample} = \frac{\text{Weighing the beaker with oil – the weight of the beaker empty}}{\text{Sample weight (in grams)}} \times 100$$

Results and discussion

The effect of the overlap between the dates of taking the readings and the treatments in reducing the number of spores of Nosema disease in infected honey bees using natural extracts:

The results of Table (1) showed that there were differences in the number of spores before and after the spraying process in the studied treatments, as the highest percentage of the number of spores was recorded in the comparison treatment before the treatment with natural extracts, especially with the sugar solution and the paste provided for feeding, with an average of 2966.50 and 2956.40, respectively. The gradual decrease in the number of spores in the studied treatments with the continuation of giving therapeutic doses, as the treatment of the alcoholic extract and by addition with the protein paste recorded the lowest number of spores after 56 days of treatment with an average of 214.20 compared with other treatments, followed by the treatment of the aqueous extract at the same date of taking the readings with an average of 456.20. The results of the statistical analysis, according to the Dunkin's multinomial test at the level of 5% probability, showed that there were significant differences between the general average of the treatments, as the alcoholic and water extract treatments excelled in reducing the number of spores and recorded the lowest average of 1359.52 and 1723.57, respectively, while the comparison treatment recorded the highest general average of 1723.57. 2885.25.

The results of the study showed that all the materials used and their treatments led to a reduction in the growth and development of Nosema spores, especially the treatment of the alcoholic extract when added with the protein paste, where the infected bees were fed on the treatment for the longest possible period of time, and this is consistent with what was found by Mura and others (2020) in the results obtained. Thereupon, while using the alcoholic extract in the treatment of infected bees, which had a very clear effect on the growth and development of Nosema spores and on their presence in the intestines of bees, especially the alcoholic extract when added with feeding.

Table 1. The average number of Nosema spores in worker bees treated with medicinal materials.

transactions	Treatment method	The number of spores before treatment	The time period for taking readings after treatment				The overall average of the treatment method	The overall average of the transaction materials
			14 days	28 days	42 days	56 days		
Control	with sugar solution	2966.50 b	2911.40 b c	2894.40 a - c	2919.30 a - c	2988.70 a	2936.06 a	2885.25 a
	With protein dough	2956.40 a b	2895.40 c	2841.10 b d	2567.70 c d	2911.60 b c	2834.44 b	
aqueous extract	with sugar solution	2865.30 a - d	2335.80 c - f	1695.40 d - f	1196.90 g - i	991.30 k	1816.94 c	1723.57 b
	With protein dough	2799.40 b – e	2232.20 f g	1556.40 f - h	997.90 k l	565.11 m	1630.20 d	
alcoholic extract	with sugar solution	2764.30 b - f	1997.80 f	1256.80 g h	997.70 k l	456.20 mn	1494.56 e	1359.52 c
	With protein dough	2711.40 d - f	396.301 e f	1123.60 i k	676.90 j - l	214.20 n	1224.48 f	
The overall average of the readings		2843.88 a	2394.81 b	1894.61 c	1559.4 d	1354.51 e		

Numbers with similar letters are not significantly different according to Dunkin's multiple range test at the probability level of 0.05%.

The effect of therapeutic materials (aqueous and alcoholic extract of propolis) on the physiological state of bees

1- The effect of therapeutic materials on the life of workers kept in cages

It was clear from the results of the death rate experiment that it differed according to the different therapeutic materials used to reduce Nosema infection. The results of the statistical analysis showed a significant difference and a clear effect on the average age of the detained worker. Table (2) showed that feeding the workers with the alcoholic extract of Borbolus with the protein paste led to an increase in the length of the age of the workers was significant compared with the comparison treatment and other treatments, as the average age of the worker was 50.10 days at the first reading, i.e. 14 days after the treatment, while we notice a difference between the two treatments of feeding with alcoholic extract and feeding with aqueous extract at the same reading, as it reached 30.77 days for the aqueous extract and the same feeding method, and the ratios between treatments varied according to the treatment method and the date of taking the readings, and reached the lowest in the comparison treatment after 56 days, and in the two methods of treatment with sugar solution and with protein dough, with an average of 21.10 and 21.20, respectively. The highest general mean for the treatments was when feeding with the alcoholic extract of borboleum with the paste, followed by feeding with the aqueous extract, with an average of 37.43 and 27.26 days, respectively,

Also, the highest general average of the readings was after 14 days of treatment, which reached 32.43 days, and the readings gradually decreased according to the time period. Nectar and Pollen, and Suwannapong et al. (2018) indicated that the type of food provided has a clear effect on the longevity of honey bees as well as its effect on its immune response to infection, and this is consistent with what Crailsheim (2012) mentioned that honey bees have the ability to survive for a long time From time to time for a long period of time on 50% (weight / volume) of protein and sugar feed to ensure the normal development of internal organs and glands as well as an appropriate immune response because it is to provide an integrated diet that includes carbohydrates and proteins as well as nutrients such as vitamins and various minerals all of which are recommended to keep bees honey in the laboratory.

Table 2. Effect of therapeutic materials (aqueous and alcoholic extract of borbulus) on the age of workers kept in cages.

transactions	Treatment method	The time period for taking readings after treatment				The overall average of the treatment method	The overall average of the transaction materials
		14 days	28 days	42 days	56 days		
Control	with sugar solution	30.00 e f	28.50 fg	25.75 e - g	24.85 e - g	27.27 c	27.26 b
	With protein dough	30.77 d e	29.10 d - f	26.10 g	23.10 h	27.26 c	
aqueous extract	with sugar solution	33.10 a - c	31.10 b - d	29.77 e f	24.10 e - g	29.51 b	37.43 a
	With protein dough	50.10 a	47.10 b	41.10 c d	43.10 b c	45.35 a	
alcoholic extract	with sugar solution	25.30 d - f	24.10 g	22.19 f g	21.10 i	23.17 d	23.26 c
	With protein dough	25.35 d - f	24.10 g	22.78 e - g	21.20 i	23.35 d	
The overall average of the readings		32.43 a	30.66 b	27.94 c	26.24 d		

Numbers with similar letters are not significantly different according to Dunkin's multiple range test at the probability level of 0.05%.

2- The effect of medicinal substances (aqueous and alcoholic extract of borbulus) on rectal pH

The results of Table (3) indicated that the acidity values of the contents of the rectum ranged between (4.11 and 5.85 PH) after treatment for 56 and 14 days, respectively, and at a temperature (25-30 C) and relative humidity (70-75%) for bees kept in cages. It reached the highest percentage of rectal acidity at the first reading after 14 days of treatment and for all treatments, especially the comparison treatment when feeding with protein dough, which did not differ significantly from sugary feeding at the same time and treatment, at a rate of 8.85 and 5.80%, respectively, while the percentages decreased continuously. The readings were taken and reached the lowest in the treatment of the alcoholic extract with the protein paste after 56 days with a pH of 4.11%. .61 and 5.59%, respectively. As for the general average of the readings, the first reading excelled after 14 days, with a pH of 5.57, which differed significantly from the rest of the readings, while the lowest rectal pH was recorded after 56 days of treatment, which was PH 4.75.

Table 3. Percentage of rectal pH in the body of bees fed therapeutic substances.

transactions	Treatment method	The time period for taking readings after treatment				The overall average of the treatment method	The overall average of the transaction materials
		14 days	28 days	42 days	56 days		
Control	with sugar solution	5.80 a	5.35 b c	4.85 b - d	4.65 c – e	5.16 b	5.19 a
	With protein dough	5,85 a	5,31 b c	4,97 b - d	4,75 c – e	5.22 a	
aqueous extract	with sugar solution	5,59 a b	5,35 b	4,95 d	4,99 c d	5.22 a	5.1 b
	With protein dough	5,61 a b	5,17 a - c	4,98 b - d	4,18 c d	4.98	
alcoholic extract	with sugar solution	5,64 a b	5,37 b	4,99 c d	4,51 c	5.12 a b	4.89 c
	With protein dough	4,95 c – e	4,87 d e	4,71 b – e	4,11 e	4.66 c	
The overall average of the readings		5.57 a	5.23 b	4.90 c	4.53 d		

Numbers with similar letters are not significantly different according to Dunkin's multiple range test at the probability level of 0.05%.

As for the general average of the treatments, the statistical analysis showed closeness of proportions between the different treatments according to Dunkin's multiple test at the probability level of 5%, as the alcoholic extract recorded the lowest percentage of rectal acidity, which was PH 4.89, followed by the water extract treatment with its percentage of PH 5.1, which did not differ from the degree of the rectal acidity of the control treatment, which recorded the highest percentage, reached PH 5.19. The reason for the low rectal pH rate may be attributed to the type of treatment provided, the method of administration, and the age of the workers. Al-Sayegh (1988) and Al-Obeidi (2021) confirmed that the rectal acidity values of caged bees ranged between (4.10-6.5 PH) and were affected by the type of food provided to worker bees and the method of feeding, while Mohammadian and others (2019) indicated that the acidity values ranged between (4.5-5.5 PH) in 30% of the individuals in the active season. In January and February, the acidity values ranged between (6.1-6.5 PH) in more than 30% of the bees. The microorganisms inside the stomach play an important role in the changes rectum.

3-The effect of therapeutic materials on the percentage of moisture content of the body of bees

The moisture content of the bee's body can give evidences of its endurance of low temperatures in winter. The lower this content was , the greater the degree of tolerance of the bee to cold could be. It is clear from Table (4) that there is the lowest moisture content in the body of bees fed with the therapeutic substance alcoholic extract of propolis with sugar feeding after 14 days, the were day of treatment, which did not significantly differ from feeding the extract with the protein dough, with a percentage of 53.25 and 53.97%, respectively.

The percentages varied according to the transactions and readings and reached the highest in the comparison treatment after 56 days with a rate of 89.74%. As for the general average of the readings, the first reading after 14 days of the treatment recorded the lowest rate of 62.31%. 73.35%, and the statistical analysis showed that there were significant differences in the general average of the treatments and according to the Dunkin's multinomial test at the probability level of 5%, as the comparison treatment recorded the highest moisture content, followed by the aqueous extract of propolis, and then the alcoholic extract, with rates of 74.8, 69.15, and 58.37%, respectively.

Table 4. Percentage of body moisture content of bees fed with therapeutic substances.

transactions	Treatment method	The time period for taking readings after treatment				The overall average of the treatment method	The overall average of the transaction materials
		14 days	28 days	42 days	56 days		
Control	with sugar solution	63,27 g	67,25 d	71,28 b - d	73,21 b – e	68.75 c	74.8 a
	With protein dough	75,93 a b	78,81 b	78,95 b	89,74 a	80.85 a	
aqueous extract	with sugar solution	59,86 g h	65,98 e f	70,39 e f	72,81 b – e	67.26 d	69.15 b
	With protein dough	67,61 c d	68,29 c	72,36 b c	75,90 a - c	71.04 b	
alcoholic extract	with sugar solution	53.25 fg	56.98 b – e	59.69 c – e	63.95 d e	57.71 f	58.37 c
	With protein dough	53,97 f g	57,93 b – e	59,71 c – e	64,52 d e	59.03 e	
The overall average of the readings		62.31 d	65.86 c	68.73 b	73.35 a		

Numbers with similar letters are not significantly different according to Dunkin's multiple range test at the probability level of 0.05%.

4-The effect of medicinal substances on the total fat content in the body of bees

The results of Table (5) showed that the lowest levels of total fat content were recorded at the start of the experiment after two weeks and for all treatments, and it was the lowest in the comparison treatment, 2.50%.

Table 5. Percentage of fat content in the body of the bee fed with therapeutic substances.

transactions	Treatment method	The time period for taking readings after treatment				The overall average of the treatment method	The overall average of the transaction materials
		14 days	28 days	42 days	56 days		
Control	with sugar solution	2.50 j	4.75 - هi	5.10 h i	5.95 h	4.57 e	4.62
	With protein dough	2.25 i j	4.50 i	4.99 h i	6.97 g h	4.67 e	c
aqueous extract	with sugar solution	7.25 g	10.40 e f	11.50 d	18.60 c d	11.93 d	12.72
	With protein dough	7.95 f g	11.50 d - f	12.95 d e	21.70 b c	13.52 c	b
alcoholic extract	with sugar solution	7.50 d - g	11.90 c – e	21.95 c	26.60 a b	16.98 b	28.69
	With protein dough	11.90 d e	26.60 a b	41.50 b	81.60 a	40.4 a	a
The overall average of the readings		6.55 d	11.60 c	16.33 b	26.90 a		

Numbers with similar letters are not significantly different according to Dunkin's multiple range test at the probability level of 0.05%.

After feeding, age progression, the length of time and the continuation of taking readings, a gradual increase in the average percentage of fat content was noted in all treatments, and reached the highest level in the treatment of the alcoholic extract of propolis with the protein paste after 56 days, at a rate of 81.60%, which surpassed all other treatments, according to Dunkin's multiple test. The limits, compared to its ratio with the aqueous extract of propolis at the same reading and treatment, amounted to 21.70%. As for the general average of the readings, the results of the statistical analysis showed, according to Dunkin's multinomial test at the level of probability 5%, a high percentage of fat content at the last reading after 56 days, that amounted to 26.90%. which was significantly superior to the rest of the averages of the readings, compared with the lowest percentage of fat content, which was recorded after 14 days of treatment, at a rate of 6.55%.

As for the general average of the treatments, the statistical analysis showed that the highest percentage of fat content was in the alcoholic extract treatment with an average of 28.69%, which significantly differed from the water extract treatment at a rate of 12.72%, while the comparison treatment recorded the lowest rate of 4.62%. The variation in the percentage of fat content is the difference in the composition of propolis after mixing it with alcohol or water and the difference in the way of feeding as well as the difference in conditions for bees kept inside cages from bees in external sects or the reason for the long period of feeding on the therapeutic substance mixed with the protein dough, when the bee food is rich in sugary substances Al-Sayegh (2000) found in his study during the period from October to mid-November that the average percentage of fat decreased to 41.9% and noted that the highest percentage of fat reached 50% in day-old workers. One and decreased to 30.77% for workers at the age of 15 days, which explains the effect of the environment and food on the fatty contents of the bee's body, and the average decreased to 27.77% in workers at the age of 3 days only.

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