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Natural Therapeutic Nutrition and its Effect on Honey Bee *Apis mellifera* L. Colonies

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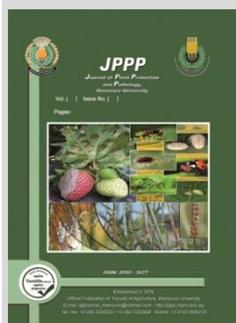
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ABSTRACT

Honey bees are one of the most important living organisms on earth due to their importance in pollinating various crops that are important to humans, in addition to producing many therapeutic products that are also important for human health. Therefore, it was important to take care of working on providing it with appropriate food to obtain protein, carbohydrates, and the types of vitamins that are important for it especially in dearth period. Here, the three therapeutic natural materials as *Thymus vulgaris*, *Cinnamomum verum* and *Syzygium aromaticum* were used mixed with supplementary diet and syrup sugar solution in the four experimental seasons (autumn, winter, spring and summer). The results reported that the highest amount of consumption was 211.50 ± 22.75 g in spring season for supplementary mixed with cinnamon and lowest amount of consumption was 107 ± 10.48 g in summer season for supplementary mixed cloves. The highest number of sealed brood was 1721 ± 191 cell and laid eggs was 2530 ± 348 egg in summer in colonies provided with supplementary mixed with cinnamon. The correlation between the consumption and the producer of sealed brood and laid eggs were 97% and 100% respectively in colonies provided by supplementary mixed with cloves. The highest amount of bee honey were 15.36 ± 1.37 kg/colony provided with supplementary with cinnamon. There were no diseases in the colonies provided with diet treated with thyme, cinnamon and cloves compared with control colonies untreated. Through this study concluded that the natural therapeutic nutrition was very useful and important to protect colonies from diseases plus increased the colonies production.

Keywords: *Thymus vulgaris*, *Cinnamomum verum*, *Syzygium aromaticum*, therapeutic supplementary diets, honeybee products.



INTRODUCTION

The health, fertility and viability of the animals are entirely dependent on the quantity and quality of food that is consumed. As for the honey bees, they obtain all their nutritional needs from pollen, nectar and water, which are collected by the honey bee workers foraging from the environment surrounding the colonies. The worker honey bees provide the colonies with the proteins, nutrients and carbohydrates they need to build the colony and feed the brood (Wright *et al.* 2018). Periods of nectar and pollen scarcity affect very negatively the activity of honey bee colonies, which leads to beekeepers resorting to feeding honey bee colonies on pollen alternatives because they believe that they suffer from nutritional deficiency (Sihag and Gupta, 2011, Pande and Karnatak, 2014 and Manning, 2016).

The supplementary diet food was a very important for the honey bee colonies to supply them by the proteins and vitamins especially in the period of time there were not pollen and nectar. The protein supplement foods used to feed honey bee colonies are not a complete substitute for natural pollen; However, using several additional products such as brewer's yeast, wheat, and soybean flours separately or mixed together improves the feeding of the colonies at a time of natural pollen scarcity. Also, to compensate for the absence of nectar and honey for bees, it is possible to use cane sugar or corn syrup to supplement bee food (Standifer *et al.* 1977).

Danelik *et al.* (2018) demonstrated it by examining changes in immune system characteristics in honey bees through different diets of pollen substitutes. And through their findings on the importance of protein content in the honey bee diet and its practical consequences for rethinking the importance of protein supplementation in a sugar-based diet and its impact on the health of honey bee colonies and the ability to survive in the winter season. Nutrition has a significant impact on the health of living organisms, their resistance to various diseases, and their ability to survive for a long time. At the global level, honey bee nutrition is an important issue for beekeepers to keep bees in good condition (Pudasaini *et al.*, 2020).

Honey is responsible for providing honey bees with the necessary energy, while pollen is responsible for supplying honey bees with important protein to feed queens and encourage them to lay eggs and also for the growth of larvae and thus increase the number of bees within colonies (Oskay, 2021). The absence of food stored inside honey bee colonies indicates their weakness, and this leads to attacking them with many diseases and pests, and in the end they are lost (Sharaf El-Din and Abd Al-Fattah, 2022). A protein supplemental diet is very important in the spring, as colonies fed by diet are more active than colonies not provided with diet supplementation. When colonies were supplemented with a protein diet in early spring, they were more active before the queen's mating season and healthier than others not

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supplemented. Supplemented food consumption was an important predictor of its quality (Shelley *et al.* 2022).

Pollen substitute feeding is provided in the form of a pancake-like paste inside honey bee colonies. This helps the young honey bees to feed and rise the brood rearing. Therefore, beekeepers use biological measurements and measurements of the growth of the bee colony to indicate the possibilities of colony breeding for queens and conducting mating operations for them (Sheesley *et al.* 1970 and Goodrich *et al.* 2019). Diets containing natural honey bee pollen have a very positive effect on the performance of honey bee colonies (Vincent *et al.* 2022).

The addition of medicinal and herbal plants to pollen substitutes increases the metabolism of honey bees and thus increases the bees' resistance to various pests and diseases (Grela *et al.*, 1998 and Pohorecka, 2004). Pollen substitutes were mixed with some medicinal herbal extracts, and camel urine and camel milk were mixed with the sugar solution. The result was a significant effect on increasing honey bee colonies, longevity of honey bee colonies, and also producing a large amount of honey and pollen (Eshbah *et al.* 2018). The quality of food provided to feed honey bees in addition to its quantity has the greatest impact on the health and longevity of honey bee individuals (Gloria *et al.* 2016). Previous studies showed that not only pollen substitutes that are used in periods of absence of pollen and nectar, such as the fall season, stimulate the survival and growth of colonies, but it is also important to pay attention to their quality and method of application (Kumar *et al.*, 2013, Morais *et al.*, 2013, Kumar and Agrawal, 2014, Pande *et al.*, 2015, Shehata, 2016, Adgaba *et al.*, 2020 and Ullah *et al.*, 2021).

Clove, thyme and cinnamon essential oils are widely considered antimicrobial against some types of bacteria. Also, the suggestion for using this kind of oils orally may provide some degree of protection against pathogens (Al- Mahdi *et al.* 2021). The thyme, cinnamon and cloves essential oil indicated highest effect against American and European Foulbrood diseases and indicated highest (Hassona, 2017). Supplied honey bees with such materials like cinnamon or chamomile or other herbal medical plants mixed with diet have potential effect on survival the population of honey bee colonies healthy (Rortais *et al.*, 2005, Brodschneider and Crailsheim, 2010).

In this study therapeutic materials as thyme, *Thymus vulgaris*, cinnamon *Cinnamomum verum* and cloves, *Syzygium aromaticum* were used as powder mixed with supplemental diet and syrup solution. Then honey bee colonies were fed on it during the four experimental seasons (autumn, winter, spring and summer) to measure the increase in the production of honey bee colonies by increasing the honey bee colonies sealed brood and laid eggs. At the end of the experiment, the quantity of bee honey produced from the tested honey bee colonies was measured as one of the most important honey bee products. During the experiment, colonies of honey bees were examined to find out the presence of diseases or non-existence.

MATERIALS AND METHODS

The colonies used in the experiments:

The experiments were started in autumn 2021 until summer 2022 through 12 month (year), at private apiary

located in village in Itay El-Baroad center, El- Behaira governorate. 20 colonies were used, each 5 colony for each experiment treated with thyme, cinnamon, cloves and 5 as control colonies. The colonies were investigated and checked each 15 days from began until the end to.

The natural therapeutic nutrition:

The three kinds of the therapeutic nutrition were used through the study *Thymus vulgaris*, *Cinnamomum verum* and *Syzygium aromaticum*. Used of them as a powder mixed with other contains of diets with proteins or carbohydrates dependent on the season also with the syrup sugar solution in all four seasons (12 month) through the year.

The natural nutrition mixed through the different seasons:

Autumn and spring seasons:

In this season the colonies provided with the protein diet dough which weight 250 g contains 215 g white maize flour +15 g of (*Thymus vulgaris* or *Cinnamomum verum* or *Syzygium aromaticum*) for the experimental colonies + 15 g pollen grains+ 10 g inactive yeast, then mixed well with warm honey solution to be cohesive dough. In addition introduce the syrup sugar solution to all experiment and control colonies by rate 1.5 sugar: 1 water with add of solution 5 g of (*Thymus vulgaris* or *Cinnamomum verum* or *Syzygium aromaticum*) for experimental colonies. As a compare with the control colonies, all control colonies supplemented with the same protein diet and syrup sugar solution but without any of the natural therapeutic.

Winter seasons:

Here the colonies provided by carbohydrate diet dough (candy) which weight 250 g contains 215 g sugar powder + 15g of (*Thymus vulgaris* or *Cinnamomum verum* or *Syzygium aromaticum*) for the experimental colonies + 15 g pollen grains+ 10 g inactive yeast, then mixed well with warm honey solution to be cohesive dough. In addition introduce the syrup solution to all experiment and control colonies by rate 2 sugar: 1 water with add of solution 5 g of (*Thymus vulgaris* or *Cinnamomum verum* or *Syzygium aromaticum*) for experimental colonies. As a compare with the control colonies, all control colonies supplemented with the same protein diet and syrup solution but without any of the natural

Summer season:

The cohesive dough diet the same used in autumn and spring season and the syrup solution the same except the rate of solution was 1 sugar: 1 water.

The observation and investigation of the all colonies treated and untreated:

The investigation and nutrition to colonies were each 15 days in each season. In this experiment the number of sealed brood and laid eggs were accounted, the brood diseases were noticed and investigated for all experimented colonies have diseases or not by percentage (%) . In addition, the amount of consumption rate of nutrition (diet supplementary treated and untreated) from each colony through each season was weighted and calculated. Also, in the end season the amount of bee honey were collected and weighted from each colony treated by (thyme, cinnamon and cloves) and untreated colonies as comparing the important product inside colonies.

Statistical analysis:

The program of SPSS 26 was used, with one way ANOVA, to analysis experimental data, through it calculated

the mean of consumption amount weight of different treated diet by g and sugar syrup by liter. in each experiment season and colonies. After that, calculated mean number of sealed brood and egg laid for each experimental colony through the 4 seasons. The mean amount weight of bee honey for the experiment seasons with different treatments calculated after the end of experiment, then Calculated *F* value, *p*. value and L.S.D. In addition, the equations of the relation between the consumption of the natural nutrition diet and the colonies products and calculated the correlation coefficient (*r*), *R* square (*R*²) and *R*² *adj.* to see the effects of the consumption of food on the sealed brood and egg laid number to increase the population of bees (Wagner and William 2015, and Schmuller, 2013).

RESULTS AND DISCUSSION

Results

The natural nutrition consumptions and its effects on the honey bee products (sealed brood and laid eggs):

The experimental data in table 1 illustrated that through the four experimental seasons autumn, winter, spring and summer the mean amounts of weight consumption of diet, mean numbers of sealed brood cells and mean numbers of laid eggs cells treated by natural therapeutic *Thymus*

vulgaris, *Cinnamomum verum*, *Syzygium aromaticum* and control (untreated). Results indicated that in autumn season the mean amounts of consumption for the three treated natural therapeutic (thyme, cinnamon and cloves) and control were 136.17±16.33, 157.83±18.83, 117.33±10.48 and 115.67±7.85 g/ check respectively with *F*. value = 58.85 and L.S.D = 7.44; also the mean amounts of sugar syrup consumption were 1, 1.5, 1 and 1.5 liter /check respectively. The mean numbers of sealed brood cells for thyme, cinnamon, cloves and control were 147±83, 392±218, 133±70 and 130±65 cell/ check respectively with *F*. value = 30.98 and L.S.D = 66.56. The mean numbers of laid eggs were 376±215, 1088±403, 336±207 and 255±125 egg/ check with *F*. value = 66.96 and L.S.D = 136.70. In addition results in winter season for the three natural therapeutic and control indicated that the mean amounts of consumption of diet were 173±22.64, 206.67±23.06, 159.00±18.07 and 157.00±18.13 g/check respectively with *F*. value = 37.42 and L.S.D = 10.87 also with the mean amount of sugar syrup solution the colonies provided with it as 2 liter /check for all treatments colonies and untreated colonies. The mean numbers of sealed brood in winter season for thyme, cinnamon, cloves and control colonies were 323±51, 739±81, 270±21 and 266±22 cell/check with *F*. value = 621.81 and L.S.D = 26.47.

Table 1. The natural nutrition feeding (g) and its effects on the colonies products (sealed brood and laid eggs) in different seasons

Experiment seasons	Natural nutrition	Amount of sugar syrup (L)/check	A mount of diet consumption (g)/check	No. of Sealed brood	No. of laid eggs
Autumn	<i>Thymus vulgaris</i>	1	136.17±16.33 ^b	147±83 ^b	376±215 ^b
	<i>Cinnamomum verum</i>	1.5	157.83±18.83 ^a	392±218 ^a	1088±403 ^a
	<i>Syzygium aromaticum</i>	1	117.33±10.48 ^c	133±70 ^{bc}	336±207 ^{bc}
	Control	1.5	115.67±7.85 ^d	130±65 ^c	255±125 ^c
	<i>F. value</i>		58.85	30.98	66.96
	<i>L.S.D</i>		7.44	66.56	136.70
Winter	<i>Thymus vulgaris</i>	2	173±22.64 ^b	323±51 ^b	857±63 ^b
	<i>Cinnamomum verum</i>	2	206.67±23.06 ^a	739±81 ^a	1722±199 ^a
	<i>Syzygium aromaticum</i>	2	159.00±18.07 ^c	270±21 ^c	790±69 ^c
	Control	2	157.00±18.13 ^c	266±22 ^c	554±58 ^d
	<i>F. value</i>		37.42	621.81	609.60
	<i>L.S.D</i>		10.87	26.47	59.94
Spring	<i>Thymus vulgaris</i>	2.5	178±22.46 ^b	706±169 ^b	1240±170 ^b
	<i>Cinnamomum verum</i>	3	211.50±22.75 ^a	1114±191 ^a	2024±282 ^a
	<i>Syzygium aromaticum</i>	2.5	164.00±18.07 ^c	647±175 ^c	1185±186 ^{bc}
	Control	2.5	162.00±18.13 ^c	662±199 ^{bc}	944±177 ^d
	<i>F. value</i>		37.47	43.97	151.13
	<i>L.S.D</i>		10.83	97.28	110.37
Summer	<i>Thymus vulgaris</i>	3	125±16.61 ^b	1199±181 ^c	1723±177 ^{bc}
	<i>Cinnamomum verum</i>	3.5	147.83±18.83 ^a	1721±191 ^a	2530±348 ^a
	<i>Syzygium aromaticum</i>	3	107.33±10.48 ^c	1206±164 ^{bc}	1746±163 ^b
	Control	3	105.83±7.99 ^c	1291±223 ^b	1542±180 ^d
	<i>F. value</i>		57.28	50.71	109.55
	<i>L.S.D</i>		7.49	100.91	121.62

The mean numbers of the laid eggs were 857±63, 1722±199, 790±69 and 554±58 egg/check with *F*. value = 609.60 and L.S.D = 59.94. In the third experimental season, spring, the mean amounts of diet consumption were 178±22.46, 211.50±22.75, 164.00±18.07 and 162.00±18.13 g/check respectively with *F*. value = 37.47 and L.S.D = 10.83. Also, the mean amounts of sugar syrup solution introduced to colonies were 2.5, 3, 2.5 and 2.5 liter/check respectively. The mean numbers of sealed brood were 706±169, 1114±191, 647±175 and 662±199 cell/check respectively with *F*. value = 43.97 and L.S.D = 97.28. The mean numbers of laid eggs were 1240±170, 2024±282, 1185±186 and 944±177 egg/check respectively with *F*. value = 151.13 and L.S.D = 110.37. Moreover, in the fourth experimental season,

summer, the mean amounts of diet consumption were 125±16.61, 147.83±18.83, 107.33±10.48 and 105.83±7.99 g/ check respectively with *F*. value = 57.28 and L.S.D = 7.49, also the mean amount of sugar syrup were 3 liter/ check for each colony treated with thyme, cloves and control colonies otherwise were 3.5 liter/check for colonies treated with cinnamon. The mean number of sealed brood for thyme, cinnamon, cloves and control colonies were 1199±181, 1721±191, 1206±164 and 1291±223 cell/check respectively with *F*. value = 50.71 and L.S.D = 100.91. The mean number of laid eggs were 1723±177, 2530±348, 1746±163 and 1542±180 egg/check respectively with *F*. value = 109.55 and L.S.D = 121.62.

The relationship between the consumption of natural nutrition therapeutic and honey bee colonies products (sealed brood and laid eggs)

In Table 2 the results were indicated the exponential equations to clarify the consumption rate effect from the diet

mixed with the various natural therapeutic materials (thyme, cinnamon and cloves) on increasing the population inside colonies as a products (sealed brood and laid eggs) through the 4 experimental seasons.

Table 2. The relation between the consumption of natural nutrition and the products in side colonies (sealed brood & laid eggs) through deferent seasons

Seasons	Independent variable		Equation*	r	R ²	F. value	P. Value	
	Natural nutrition	Products						
Autumn	<i>Thymus vulgaris</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 8.661 + 0.020 \text{ B}$ (1.384) (3.736)	0.577	0.333	13.955	0.001	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 17.457 + 0.021 \text{ E}$ (13.912) (0.006)	0.569	0.324	13.421	0.001	
	<i>Cinnamomum verum</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 18.211 + 0.18 \text{ B}$ (1.254) (3.666)	0.569	0.324	13.437	0.001	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 112.437 + 0.014 \text{ E}$ (2.249) (4.984)	0.686	0.470	24.836	0.000	
	<i>Syzygium aromaticum</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 1.345 + 0.038 \text{ B}$ (1.173) (5.235)	0.703	0.495	27.406	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 1.594 + 0.044 \text{ E}$ (1.006) (5.205)	0.701	0.942	27.097	0.000	
	Control	Sealed brood	$\text{Ln } \hat{Y}_1 = 0.645 + 0.045 \text{ B}$ (0.862) (4.458)	0.644	0.415	18.876	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 1.078 + 0.046 \text{ E}$ (0.918) (4.911)	0.680	0.463	24.114	0.000	
	Winter	<i>Thymus vulgaris</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 148.210 + 0.004 \text{ B}$ (5.668) (4.383)	0.638	0.407	19.215	0.000
			Laid eggs	$\text{Ln } \hat{Y}_2 = 680.504 + 0.001 \text{ E}$ (10.257) (12.353)	0.406	0.165	5.535	0.026
		<i>Cinnamomum verum</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 452.326 + 0.002 \text{ B}$ (6.067) (2.960)	0.488	0.238	8.762	0.006
			Laid eggs	$\text{Ln } \hat{Y}_2 = 1126.483 + 0.002 \text{ E}$ (5.677) (2.388)	0.411	0.169	5.701	0.024
<i>Syzygium aromaticum</i>		Sealed brood	$\text{Ln } \hat{Y}_1 = 140.781 + 0.004 \text{ B}$ (31.311) (20.429)	0.968	0.937	417.346	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 431.811 + 0.004 \text{ E}$ (9.938) (6.001)	0.750	0.563	36.017	0.000	
Control		Sealed brood	$\text{Ln } \hat{Y}_1 = 135.252 + 0.004 \text{ B}$ (23.346) (15.786)	0.948	0.899	249.209	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 253.789 + 0.005 \text{ E}$ (9.315) (7.265)	0.808	0.653	52.785	0.000	
Spring		<i>Thymus vulgaris</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 129.678 + 0.009 \text{ B}$ (5.313) (8.923)	0.860	0.740	79.619	0.000
			Laid eggs	$\text{Ln } \hat{Y}_2 = 515.017 + 0.005 \text{ E}$ (8.024) (7.028)	0.799	0.638	49.399	0.000
		<i>Cinnamomum verum</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 277.595 + 0.007 \text{ B}$ (5.969) (8.253)	0.842	0.709	68.112	0.000
			Laid eggs	$\text{Ln } \hat{Y}_2 = 1762.542 + 0.001 \text{ E}$ (3.983) (0.515)	0.097	0.009	0.265	0.610
	<i>Syzygium aromaticum</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 51.200 + 0.015 \text{ B}$ (8.168) (20.522)	0.968	0.938	421.353	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 275.598 + 0.009 \text{ E}$ (43.351) (63.068)	0.996	0.993	3977.615	0.000	
	Control	Sealed brood	$\text{Ln } \hat{Y}_1 = 49.593 + 0.016 \text{ B}$ (4.693) (12.020)	0.915	0.838	144.472	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 169.526 + 0.010 \text{ E}$ (21.647) (37.013)	0.990	0.980	1369.996	0.000	
	Summer	<i>Thymus vulgaris</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 681.272 + 0.004 \text{ B}$ (5.188) (2.898)	0.480	0.231	8.398	0.007
			Laid eggs	$\text{Ln } \hat{Y}_2 = 1283.497 + 0.002 \text{ E}$ (7.265) (2.118)	0.372	0.138	4.488	0.043
		<i>Cinnamomum verum</i>	Sealed brood	$\text{Ln } \hat{Y}_1 = 911.030 + 0.004 \text{ B}$ (8.523) (5.412)	0.715	0.511	29.291	0.000
			Laid eggs	$\text{Ln } \hat{Y}_2 = 2993.041 + 0.001 \text{ E}$ (4.722) (-0.846)	0.158	0.025	0.715	0.405
<i>Syzygium aromaticum</i>		Sealed brood	$\text{Ln } \hat{Y}_1 = 485.234 + 0.008 \text{ B}$ (5.012) (4.540)	0.651	0.424	20.608	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 923.290 + 0.006 \text{ E}$ (7.259) (4.614)	0.657	0.432	21.288	0.000	
Control		Sealed brood	$\text{Ln } \hat{Y}_1 = 196.437 + 0.018 \text{ B}$ (4.032) (7.554)	0.819	0.671	57.062	0.000	
		Laid eggs	$\text{Ln } \hat{Y}_2 = 437.571 + 0.012 \text{ E}$ (5.612) (7.050)	0.800	0.640	49.707	0.000	

R² = coefficient of determination, r = correlation coefficient, numbers between () is t calculated

The thyme, *Thymus vulgaris*, in the first experimental season (autumn) when the colonies consumption one gram from the diet mixed with that material the sealed brood increased by 0.02 cell from the population with *F. value* = 13.955, *P. value* = 0.001, and calculated the correlation coefficient (*r*) was 0.577 and coefficient of determination R^2 was 0.333. Also, when bees inside colonies feed and consumption 1 gram from the mixed which lead to laid eggs increased by 0.021 egg from the population with *F. value* = 13.421, *P. value* = 0.001, *r* = 0.569 and R^2 = 0.324. In winter season when the bees consumption 1 gram of mixed diet by thyme the sealed brood increased by 0.004 with *F. value* = 19.215, *P. value* = 0.000, *r* = 0.638 and R^2 = 0.407, also the laid eggs increased by 0.001 with *F. value* = 5.535, *P. value* = 0.026, *r* = 0.406 and R^2 = 0.165. In the third season which was spring the consumption 1 gram from the mixed diet with thyme effected on the sealed brood to increase it by 0.009 with *F. value* = 79.619, *P. value* = 0.000, *r* = 0.860 and R^2 = 0.740. Moreover, the laid eggs increased by 0.005 with *F. value* = 49.399, *P. value* = 0.000, *r* = 0.799 and R^2 = 0.638. In summer season the bees feed and each 1 gram they consumption from the mixed diet with thyme effected on the sealed brood and increased it by 0.004 of population with *F. value* = 8.398, *P. value* = 0.007, *r* = 0.480 and R^2 = 0.231. The same happened with the laid eggs increased by 0.002 with *F. value* = 4.488, *P. value* = 0.043, *r* = 0.372 and R^2 = 0.138. The second experiment material mixed with diet and introduced to bees was *Cinnamomum verum* and each 1 g consumed from it in autumn season leads to increase the sealed brood by 0.18 with *F. value* = 13.437, *P. value* = 0.001, *r* = 0.569 and R^2 = 0.324. Laid eggs also increased by 0.014 with *F. value* = 24.836, *P. value* = 0.000, *r* = 0.686 and R^2 = 0.470. In the second season (winter) each 1 g consumed from the cinnamon mixed diet lead to increase the sealed brood by 0.002 with *F. value* = 8.762, *P. value* = 0.006, *r* = 0.488 and R^2 = 0.238. And laid eggs increased by 0.002 also with *F. value* = 5.701, *P. value* = 0.024, *r* = 0.411 and R^2 = 0.169. In spring the sealed brood increased by 0.007 with *F. value* = 68.112, *P. value* = 0.000, *r* = 0.842 and R^2 = 0.709, moreover the laid eggs increased by 0.007 with *F. value* = 0.265, *P. value* = 0.610, *r* = 0.097 and R^2 = 0.009. In summer, the sealed brood increased by 0.004 with *F. value* = 29.291, *P. value* = 0.000, *r* = 0.715 and R^2 = 0.511, the laid eggs increased by 0.001 with *F. value* = 0.715, *P. value* = 0.405, *r* = 0.158 and R^2 = 0.025.

Syzygium aromaticum which consumed by bees inside colonies through the diet mixed with cloves effected on the sealed brood and when bees consumed 1 g from it in autumn increased sealed brood by 0.038 with *F. value* = 27.406, *P. value* = 0.000, *r* = 0.703 and R^2 = 0.495, and laid eggs increased by 0.044 with *F. value* = 27.097, *P. value* = 0.000, *r* = 0.701 and R^2 = 0.942. In winter, the sealed brood increased by 0.004 with *F. value* = 417.346, *P. value* = 0.000, *r* = 0.968 and R^2 = 0.937, also the laid eggs increased by 0.004 with *F. value* = 36.017, *P. value* = 0.000, *r* = 0.750 and R^2 = 0.563. In spring as the third season the sealed brood increased by 0.015 with *F. value* = 421.353, *P. value* = 0.000, *r* = 0.968 and R^2 = 0.938, and laid eggs increased by 0.009 with *F. value* = 3977.615, *P. value* = 0.000, *r* = 0.996 and R^2 = 0.993. In the summer season, the sealed brood increased by 0.008 with *F. value* = 20.608, *P. value* = 0.000, *r* = 0.651 and R^2 = 0.424, and laid eggs increased by 0.006 with *F. value* = 21.288, *P. value* = 0.000, *r* = 0.657 and R^2 = 0.432.

As a comparison with the control diet consumed 1 g from it in autumn leads to the sealed brood increased by 0.045 with *F. value* = 18.876, *P. value* = 0.000, *r* = 0.644 and R^2 = 0.415, and laid eggs increased by 0.046 with *F. value* = 24.114, *P. value* = 0.000, *r* = 0.680 and R^2 = 0.463. Also, in winter the sealed brood increased by 0.004 with *F. value* = 249.209, *P. value* = 0.000, *r* = 0.948 and R^2 = 0.899, and laid eggs increased by 0.005 with *F. value* = 52.785, *P. value* = 0.000, *r* = 0.808 and R^2 = 0.653. In spring, sealed brood increased by 0.016 with *F. value* = 144.472, *P. value* = 0.000, *r* = 0.915 and R^2 = 0.838, and laid eggs increased by 0.010 with *F. value* = 1369.996, *P. value* = 0.000, *r* = 0.990 and R^2 = 0.980. In summer as the end season of this experiment, sealed brood increased by 0.018 with *F. value* = 57.062, *P. value* = 0.000, *r* = 0.819 and R^2 = 0.671, and laid eggs increased by 0.012 with *F. value* = 49.707, *P. value* = 0.000, *r* = 0.800 and R^2 = 0.640.

Bee honey as the mean product from the colonies treated after year (4 seasons) experiment:

Through figure (1) illustrated that the mean of bee honey weight after 12 month (4 seasons) after the experiment of natural nutrition therapeutic with the four materials finished their experiment. Collected all amount of bee honey from each treated and control colonies then weighted all bee honey from each colony treated and untreated, the results reported in the table 3 were, the mean of bee honey weight by kg from the 5 colonies had the nutrition (diet and syrup sugar) mixed with thyme *Thymus vulgaris* was 12.96 kg/colony. In addition the mean weight of the bee honey collected from the 5 colonies had nutrition mixed with cinnamon *Cinnamomum verum* was 15.36 kg/colony. Moreover, the mean weight of bee honey collected from the other 5 colonies had nutrition mixed with cloves *Syzygium aromaticum* was 11.56 kg/colony. Finally, the mean weight of bee honey collected from the 5 colonies as control had untreated nutrition was 10.65 kg/colony, with *F. value* = 21.220, *P. value* = 0.000 and L.S.D = 1.436. Though the data the statistical analysis indicated that there were significant different between the weights of bee honey from each colonies according to the kind of nutrition their consumed.

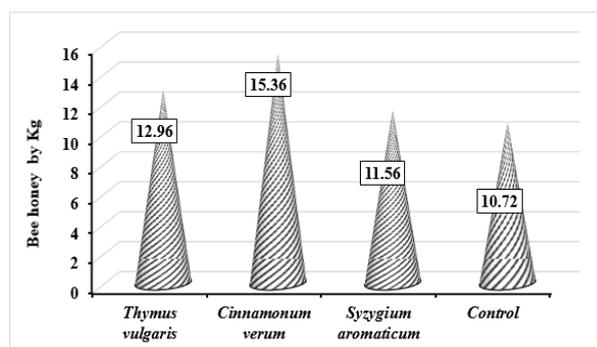


Figure 1. The mean amount of bee honey product from the colonies after (4 seasons) the end of natural nutrition experiment

Investigation of the diseases in the colonies treated and untreated

In table 3 indicated the three kind of diseases (American foulbrood & European foulbrood, Varroa mite, *Nosema sp.* and Chalk brood) was important to be know there were in the colonies or not. So, in the time of investigation through the experiment the data in table 3 illustrated that there

were no (0%) diseases from any kind in all colonies treated with *Thymus vulgaris*, *Cinnamomum verum* and *Syzygium aromaticum* through the 4 experiments seasons. On the other hand, there were two kinds in the disease reported in the colonies used as a control (untreated) Varroa mite appeared in all 4 seasons by 43% in autumn, 29% in winter, 15% in spring and 12% in summer seasons there were low account controlled through the experiment. Then, chalk brood appeared in autumn with 52%, in winter with 25% and in spring with 22% theses were few amounts under control, and then disappeared in summer season. Also, the *Nosema sp.* appeared in winter with 56% and spring with 44% seasons only with few amount of each season under control.

Table 3. The diseases of brood found or not in the different colonies have diet and syrup with natural therapeutic

The natural nutrition	The seasons	Types of diseases percentage (%)			
		U.F.B. and A. F.B.	Varroa mite	Nosema sp.	Chalk brood
<i>Thymus vulgaris</i>	Autumn	0	0	0	0
	Winter	0	0	0	0
	Spring	0	0	0	0
	Summer	0	0	0	0
<i>Cinnamomum verum</i>	Autumn	0	0	0	0
	Winter	0	0	0	0
	Spring	0	0	0	0
<i>Syzygium aromaticum</i>	Autumn	0	0	0	0
	Winter	0	0	0	0
	Spring	0	0	0	0
Control	Autumn	0	43	0	52
	Winter	0	29	56	25
	Spring	0	15	44	22
	Summer	0	12	0	0

Discussions

The current results indicated that the highest consumed diet treated by cinnamon were 180.85g/ season, from 250g of introduced diet, with percentage of 72.3% followed by consumed diet treated with thyme 153.04 g/season by percentage of 61.12% then diet treated with cloves 136.9g /season by percentage of 54.8% and diet used as control consumed by colonies through the 4 seasons 135.13g/ season by 54%. The result illustrated that the most nutrition consumed highest by honey bees in colonies were the diet treated with cinnamon and lowest nutrition consumed were control diet and diet treated by cloves. In general the honey bee colonies reported that the most seasons they consumed the diet supplement with treated or not was the spring season followed by winter those periods were the poor period of pollen and nectar around the experimental colonies. The biggest amount of consumption diet supplement were in spring season for the diet treated with therapeutic cinnamon *Cinnamomum verum* by 84.6% followed by winter season 82.7% . supplementary feeding was very important in the colonies specially in early spring when the pollen and nectar were not available any more that’s helped colonies to survive (Standifer *et al.* 1977). The different industrial diets that honey bees feed on for bees are usually rich in protein such as (yeast, soybeans, corn, peas, eggs, microalgae and casein) and may include a small amount of pollen because of its

importance in increasing the rate of consumption and brood rearing (Standifer *et al* 1973 and Alqarni *et al* 2006).

The consumed of diet and syrup sugar solution treated with natural therapeutic effected on the population inside colonies, the number of the sealed brood and laid eggs increased by continues of feeding from autumn to summer as the sealed brood was 147 cell in autumn increased to be 1199 cell in summer season also the laid eggs was 376 egg in autumn increased to be 1723 egg in summer season these was during continuously feeding on the diet with thyme *Thymus vulgaris*. The same happened with the colonies continuously feeding on diet with cinnamon *Cinnamomum verum* the sealed brood was 392 cells in autumn increased to be 1721 cell in summer and the laid eggs was 1088 egg in autumn increased to be 2530 egg in summer. The colonies consumed the diet mixed with cloves *Syzygium aromatic* produced sealed brood 133 cell in autumn then increased to 1206 cell in summer season, and laid eggs 336 egg in autumn and increased to 1746 egg in summer season. On the other hand, the control diet produced lowest sealed brood and eggs but also sealed brood was 130 cell in autumn increased to 1291 cell in summer, also laid eggs 255 egg in autumn increased to 1542 egg in summer season by the continuously feeding on diet and syrup solution. The supplementary diets helping surviving the colonies and increasing the bee population to produce honey and collect pollen (Standifer *et al.* 1977). Supplemented honey bee colonies with the diet protein effected in the honey bee brood and increased the percentage of brood production from 13.1 to 14.5% (Vrabie *et al.* 2019). In general supplemented food used as strategy by beekeepers to increase the honey bee individuals especially in the period has not pollen and nectar, but the supplementary food must have protein and carbohydrates (Pudasaini *et al.* 2020).

The results compared between the three diets nutrition with natural treated materials (*Thymus vulgaris*, *Cinnamomum verum* and *Syzygium aromaticum*) the colonies indicated the highest amount of consumption and highest number of sealed brood and laid eggs were colonies fed on diet treated with cinnamon *Cinnamomum verum*. On the contrary, the colonies fed on the diet treated with cloves *Syzygium aromaticum* they were recorded the least consumption and least number of sealed brood and laid eggs. Even though, the control colonies were the least one in the consumption and production of brood and eggs. Supplemented commercially diets were used in honey bee colonies leads to improvement and sustainable the brood rearing (Abd El- Wahab *et al.* 2016). The tested supplementary diets differed in their consumption by honey bees, some diets being consumed entirely compared to others (DeGrandi – Hoffman *et al.*, 2008). May be the artificial supplementary diets effective on stimulating the honey bee queen to produced eggs and also colonies to rear brood (Mattila and Otis, 2006, Nabors, 2000 and Standifer *et al.*, 1973),

Through the equation calculated to indicate the relationship between the rate of consumption and the production amount of brood and eggs, the results indicated that there were highest rate of correlation between the consumed food and the produced brood especially in spring season 86% brood and 80% eggs in colonies fed on the diet treated with thyme *Thymus vulgaris*. In addition there were 84% brood and 97% eggs in colonies fed on diet treated with cinnamon *Cinnamomum verum*, also the colonies fed on diet treated with cloves indicated highest correlation between the

consumption and production in spring season by 97% brood and 100% eggs. Alternating diet pollen and pollen only accepted from the honey bee, thus the pollen replacement diet is highly portable from honey bees as natural pollen and it can be introduced to colonies as patties (Saffari *et al.*, 2004). There was an important role in artificial supplement diets in increasing the bee honey production, brood production, other biological activity and also immunity (Skubida *et al.*, 2008, Zahra and Talal, 2008).

The supplementary food introduced to the colonies through the 4 experiments seasons by using the three different natural therapeutic materials indicated, the high amount in bee honey produced after the end of the experiment compared with the control colonies as it happened in sealed brood and laid eggs production. The highest amount of the bee honey produced was 15.36 kg /colony fed on diet and syrup treated with cinnamon *Cinnamomum verum*, and the least amount of bee honey produced was 11.56 kg/colony fed on diet and syrup treated with cloves *Syzygium aromaticum*. Otherwise, the control colony produced 10.72 kg at the end of experiment. It is necessary to provide protein feed stimulate colony activity, maximize honey production and pollination of crops, overcome pesticide damage and resist parasites and diseases, and drive bee production flow (Skubida *et al.*, 2008). Another study indicated that the higher of consumed diet than other types of diets lead to higher in honey bee population then followed by the higher in bee honey production and pollination (Kumar *et al.*, 1995).

The mains of using the three different natural therapeutic materials mixed with diet and syrup were make bees more attracted to feeding; prevent the presence of any kind of brood disease as appeared through the experiment that all brood diseases reported only in the control colonies, as *Nosema sp.*, Chalk brood appeared in winter and summer seasons and Varroa mite in all seasons. Also, using the three different natural therapeutic materials mixed with diets and syrup increasing the numbers of honey bee's population within the colonies by stimulating the queen to lay eggs, especially at a time when there is no pollen and nectar, and this in turn leads to an increase in the colonies production of honey, pollen and other important products. Authors were studied that natural materials (chamomile and cinnamon) effected on the colonies as the cinnamon prevent the Varroa mite and helping honey bees to build the wax foundation faster than chamomile group; in addition the population of bees increased more in the group of chamomile than cinnamon group, both chamomile and cinnamon indicated good effect in all biological activities and survived healthy for honey bee population (Al-Ghamdi *et al.* 2021). The materials add to supplementary diet and syrup indicated that improved the bees healthy, moreover there was no side effect appeared on the honey bees through the investigation (Rortais *et al.*, 2005, Brodschneider and Crailsheim, 2010).

By using different natural nutrition materials some effective on morphometric analysis happened as in forewings and hind legs in bees treated, these differences between the natural materials may be due to other factors such as genetic variations (Garnery *et al.*, 1998, Arias *et al.* 2006 and Marghitas *et al.* 2008).

CONCLUSION

In current study the three natural materials therapeutic were mixed with the different diets dependent on the season and compared with the control diet introduced to other

colonies. All the three materials thyme, cinnamon and cloves indicated good effect in the colonies treated. The cinnamon was much better than thyme and cloves in the bee population production and bee honey production. In addition all of them indicated perfect effect to prevent any brood diseases in all colonies treated by each of them. Still need more studies on the honey bee biological activity for the three therapeutic materials.

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التغذية العلاجية الطبيعية وتأثيرها على طوائف نحل العسل

نادية محمد خميس مصطفى حسونة

قسم وقاية النباتات كلية الزراعة سببا باشا جامعة الاسكندرية

المخلص

من خلال الدراسة تم استخدام المواد العلاجية الطبيعية الثلاثة وهي *Thymus vulgaris* الزعتر والقرفة و *Cinnamomum verum* و *Syzygium aromaticum* القرنفل، ممزوجة مع النظام الغذائي التكميلي (بدائل حيوب اللقاح) ومحلول السكر في فصول التجربة الأربعة (الخريف والشتاء والربيع والصيف). أظهرت النتائج أن أعلى كمية استهلاك كانت 22.75 ± 211.50 جم في فصل الربيع للمكملات الممزوجة بالقرفة وأقل كمية استهلاك كانت 7.99 ± 125 جم في موسم الصيف للمخلوط التكميلي الممزوج بالزعتر. كان أعلى عدد من الحضنة المختومة 191 ± 1721 عين سداسية وكان البيض الموضوع 348 ± 2530 بيضة في الصيف في الطوائف المزودة بمكملات ممزوجة بالقرفة. كان الارتباط بين الاستهلاك والإنتاج للحضنة المختومة والبيض الموضوع 97% و 100% على التوالي في الطوائف المزودة بمكملات ممزوجة بالقرنفل. وكانت أعلى كمية من عسل النحل 1.37 ± 15.36 كجم / طائفة مزودة بمكملات القرفة ومن خلال الفحوصات لاحظ عدم وجود أي نوع من أمراض الحضنة في الطوائف المزودة بنظام غذائي معالج بالزعتر والقرفة والقرنفل مقارنة بالطوائف المقارنة الغير مزودة بأي من المواد المضافة المعالجة الغذائية.

الكلمات الدالة: الزعتر، القرفة، القرنفل، بدائل حيوب اللقاح العلاجية، منتجات نحل العسل