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STUDIES ON TIME APPEARANCE AND CONTROL OF CHALKBROOD DISEASE IN MINIA REGION

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ABASTRACT

Surveying of chalkbrood disease was carried out at four locations of Minia governorate, over the year. Results showed that the highest infection percentage (٧.٢٥٪) was recorded in ٢٠ April ٢٠١٠ for chalkbrood disease which associated with average temperature ٣٢.٤ ºC and ٦٩.٦٪ relative humidity. However, the lowest infection (٠.٠٠ – ٠.٦٦٪) was in minimum level over long period extended between half of December, ٢٠٠٩ to last of March, ٢٠١٠. The prevailing temperature of the last mentioned period was ranged between ١٨.٣-٢٥.٨ ºC and relative humidity ranged between ٧٧.١-٦٥.٩٪.

Three chemicals (acetyl salicylic acid, Thymol and potassium sorbate) and one antibiotic (Terramycine) were used to control chalkbrood disease. The tested materials were used in three features (sugar powder, candy and sugar syrup). The obtained results also revealed that using acetyl salicylic acid gave the highest reduction percentages of chalkbrood infection (up to ٥٢.٥٤٪ in autumn and ٨٠.٤١٪ in winter). Terramycine came in second position which caused reduction percentages up ٣٣.٢٦٪ in autumn, and ٨٠.٠٧٪ in winter. Thymol treatment occupied the third level which reduced the infection by ٣٦.٩١٪ in autumn, and ٧٥.٠٩٪ in winter. However, using potassium sorbate didn't show satisfy efficacy against the disease, it was resulted in reduction percentages up to ٣.٣٠٪ in autumn, and ٣٧.٧٢٪ in wint

INTRODUCTION

Chalkbrood is a fungal disease of honey bee brood caused by *Ascosphaera apis*. (Douglas ١٩٩٣, Masterman *et al.* ٢٠٠١, Chorbinski ٢٠٠٤, Flores *et al.* ٢٠٠٥, Aronstein and Murray ٢٠١٠). Young honeybee larvae (٣-٤ days old) are most susceptible to chalkbrood infection (Bailey and Ball ١٩٩١). Three volatile compounds collected from larvae infected with the fungal pathogen *Ascosphaera apis* and detected by adult honey bees, were identified by coupled gas chromatography-electroantennographic detection and gas chromatography-mass spectrometry. These three compounds are phenethyl acetate, ٢-phenylethanol, and benzyl alcohol, which present in volatile components collected from infected larvae. Two field bioassays revealed that one of these compounds is phenethyl acetate, which considered a key compound associated with *Ascosphaera apis* infected larvae that induces hygienic behavior of the bee worker (Swanson *et al.* ٢٠٠٩). Lytic enzymes are usually reported to have a role in fungal entomopathogenicity. Enzymic profiles produced by *Ascosphaera apis*. Ten isolates of *A. apis* produced ١١ enzymes. Two main enzymes (protease and beta -N-acetylglucosaminidase), that might play roles in either penetration of the peritrophic membrane in bee larval midgut or breaking down the cuticle of larvae were reconized (Theantana and Chantawannakul ٢٠٠٨). This fungus germinates in the larval gut either pre- or post-capping when colony temperature falls below the optimal temperature of ٣٢–٣٥ °C for a prolonged period (>٢ h) (Bailey and Ball, ١٩٩١). Fungal infection is positively correlated with temperature and negatively correlated with relative humidity in all localities (Zidan *et al.* ١٩٩٩).

The present study aimed to record the time appearance of chalkbrood disease and testing the efficacy of certain chemicals against this disease.

MATERIALS AND METHODS

The present study was carried out at four locations of Minia governorate, Faculty of agriculture apiary of Minia university and three private apiaries (One apiary at Shosha village, Samalot district

and another two apiaries at Damares village, Minia district). Trails of the study were conducted through two successive years (September ٢٠٠٩ to August ٢٠١١).

١. Experimental Bees:

Fifty seven Carnioilan honeybees (*Apis mellifera carnica*) colonies having an approximately equal strength (bees covered ٨ wax combs), recent mated sister queens, equal stored food (honey and pollen) and about the same level of infection of chalkbrood disease were selected for the experiments of the study .

The experimental bee colonies were housed in Langstroth hives. They received normal feeding program over the period of study. Simple trap (wooden barrier) was placed on the hive entrance of each experimental bee colony to prevent the workers to throw out the dead larvae outside the hive which helped in counting number of mummies of chalkbrood (Medina and Mejia, ١٩٩٩).

٢. Scientific axis of the study:

The current study was focused on two axis as follows :-

١) Record timing of appearance of fungal diseases and its relation to temperature and relative humidity over the year.

٢) Controlling chalkbrood disease by using certain chemicals and Terramycine (antibiotic).

٢.١. Record timing of appearance of chalkbrood symptoms on honeybee larvae and its relation to temperature and relative humidity over the year:

Twelve colonies of different four experimental apiaries, three colonies each, were inspected at ١٢ days intervals over the year to record two parameters as follows :-

a) Area of the brood which measured through using wired grad frame having ١.٠٠ sq.inch divisions according to method of **De Jong, (١٩٧٦)** and number of the brood cells was calculated based on fact saying that each one square inch having ٢٥ worker hexagonal cells.

b) Number of the infected larvae with fungal diseases found in brood cells or on the bottom board of the hive.

Percentage of the infected brood cells was estimated according to the following formula:

$$\%\text{infected bee larvae} = \frac{\text{Number of infected larvae}}{\text{Number of the total brood cells}} \times ١٠٠$$

The mean percentage of infected larvae by chalkbrood disease over ١٢ day intervals was estimated. The relationship between timing of appearance of chalkbrood disease infection and the weather factors was studied as follows:-

Data of the average temperature and relative humidity over the year was got from metrological station of Minia airport. Correlation and regression coefficients between the percentage of fungal diseases infection and both temperature and relative humidity were determined.

٢.٢ Controlling chalkbrood disease by using an antibiotic and other three chemicals:

Three chemicals (acetyl salicylic acid, Thymol and potassium sorbate) and one antibiotic (Terramycine) were used for controlling chalkbrood disease as follows:-

The tested materials were mixed each with one of three types of feeding component (powdered sugar, candy and sugar syrup) by concentration described in the following table:-

Table I :Concentration and dosage of certain chemicals and an antibiotic used for controlling chalkbrood disease infected honeybee colonies.

Name	Concentration in			Dosage per colony			Reference
	Sugar powder	candy	sugar syrup	Sugar powder	candy	sugar syrup	
Acetyl salicylic acid	٠.٠١٪	٠.٠١ %	١٠٠ ppm	٤٠ gr.	٤٠ gr.	٢٥٠ ml	(Jenko et al., ١٩٩١)
Thymol	٠.٧٠ %	٠.٧٠ %	٠.٧٠ %	٤٠ gr.	٤٠ gr.	١٠٠ ml	(Davis and ward, ٢٠٠٣)
Potassium sorbate	٠.٠١٪	٠.٠١ %	١٠٠ ppm	٤٠ gr.	٤٠ gr.	٢٥٠ ml	Menapace and Hale ١٩٨١, Thurber ١٩٧٩)
Terramycine	٥.٠٠٪	٥.٠٠ %	٥.٠٠٪	٤٠ gr.	٤٠ gr.	٢٥٠ ml	(Flores et al., ٢٠٠٤)

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Sugar powder or candy having the applied chemicals were placed on plastic sheets over the top bars of the combs of the tested colonies (Rembold *et al.,* ١٩٨٠), while sugar syrup having the same chemicals was placed in Doolitle side plastic feeder.

The tested materials were supplied to the experimental bee colonies over the year at ١٢ days intervals, except the period of nectar flow (April to September). Forty five experimental bee colonies were used for that part of the study. Among that number of the colonies, nine colonies for each applied chemical (three bee colonies were specified for each feeding treatment) in addition to another nine colonies (three colonies for each feeding treatment) receiving no chemicals were acted as control sets.

٢.٢.١ Efficacy of different chemicals on infection of chalkbrood disease :-

Infestation percentages of chalkbrood were estimated in the experimental colonies at ١٢ days intervals through determination of infection percentage before and after treatment in treated colonies as well as in control set. Reduction percentage of infection was calculated through applying formula of Henderson and Telton (Henderson and Telton, ١٩٥٥) which saying that :

$$\text{Reduction \%} = ١٠٠ \left(١ - \frac{\text{infection\% after treatment} \times \text{infection\% before control}}{\text{infection\% after control} \times \text{infection\% before treatment}} \right)$$

٢.٢.٢. Statistical analysis:-

The obtained data were subjected to one way analysis of variance and the difference among means of treatments were compared according to least significant range (Duncan range) tests. Also, correlation and regression coefficients were estimated according to method of Mead et al., ١٩٩٣.

RESULTS AND DISCUSSION

١. Record timing of appearance of chalkbrood infection and its relation to temperature and relative humidity over the year:-

Appearance symptoms of chalkbrood disease was recorded in certain apiaries located in Minia region over two successive years as follows:

Data in Table (١) and Figure (١) of the first season (٢٠٠٩/٢٠١٠) showed that the highest percentage of infection of chalkbrood was associated with average temperature ٣٢.٤ ºC and ٦٩.٦٪ relative humidity. While the lowest percentage of infection was recorded with range of temperature extended between ١٤.٧ to ٢٥.٨ ºC and relative humidity ranged between ٥٩.٦ to ٦٥.٩٪. It can be identified two peaks of chalkbrood infection over the year, the first was recorded on September (٦.١٣٪), while the second one appeared on ٢٠ April (٧.٢٥٪). On contrast, the symptoms of the disease was absent over the winter period.

The same trend was recorded in the second season (٢٠١٠/٢٠١١) where the highest infection (٧.١٩٪) was observed in April with mean of temperature and relative humidity was ٣١.٩ ºC and ٧٠.٣٪, respectively. Also, chalkbrood infection was in minimum level over long period extended between half of December to last of March (٠.٠ – ٠.٧٥٪) where temperature degrees ranged between ١٨.٧ to ٢٦٥.٤ ºC and relative humidity ranged between ٧٦.٤ to ٦٦.١٪.

Determination of correlation coefficient at the first season (٢٠٠٩/٢٠١٠) between percentage of infection and both temperature and relative humidity revealed that there was positive significant correlation (٠.٧١) between infection percentage and temperature. While weak positive correlation (٠.٢٩) was existed between infection percentage and relative humidity. Similarly, the correlation coefficient at the second season (٢٠١٠/٢٠١١) between percentage of infection and both temperature and relative humidity were ٠.٦٦, ٠.٣٣, respectively.

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Table ١: Development the infection of honeybee brood by chalkbrood disease during ٢٠٠٩/٢٠١٠ and ٢٠١٠/٢٠١١ seasons at Minia region.

	Dates	%Mean Infection /colony		Mean daily temperature °C		Mean daily relative humidity%	
		٢٠٠٩/٢٠١٠	٢٠١٠/٢٠١١	٢٠٠٩/٢٠١٠	٢٠١٠/٢٠١١	٢٠٠٩/٢٠١٠	٢٠١٠/٢٠١١
autumn	١٥ September	٦.٠٩	٥.٨٩	٢٨.٢	٣٠.٢	٦٨.٤	٦٦.٩
	٢٧ September	٦.١٣	٥.٨٢	٢٩.٣	٢٨.٧	٧٠.١	٧١.٣
	٩ October	٤.٨١	٤.٥٨	٢٧.٥	٢٧.٩	٦٦.٣	٦٨.٣
	٢١ October	٣.٩٢	٣.٨٥	٢٥.٩	٢٧.٣	٦٩.٤	٦٨.٧
	٢ November	٣.٨٩	٣.٦٧	٢٣.٣	٢٢.٩	٧٢.٧	٧٠.٢
	١٤ November	٢.٧١	٢.٥٣	٢١.٨	٢٢.٦	٧٥.١	٧٤.٣
	٢٦ November	٢.١٣	٢.١١	٢٢.٢	٢١.٨	٧٣.٥	٧٢.٨
	٨ December	١.١٥	١.٠٣	١٨.٤	١٩.٣	٧٦.٤	٧٥.٨
	٢٠ December	٠.٦٦	٠.٧٥	١٨.٣	١٨.٧	٧٧.١	٧٦.٤
winter	٢ January	٠.٠٠٥	٠.٠٠٦	١٦.٢	١٦.٨	٦٧.٧	٦٦.٢
	١٤ January	٠.٠٠٣	٠.٠٠٣	١٤.٣	١٥.٧	٦١.٢	٦٣.٤
	٢٦ January	٠.٠٠	٠.٠٠	١٤.٧	١٤.٩	٥٩.٦	٦٠.٧
	٧ February	٠.٠٠	٠.٠٠	١٩.٦	٢٠.١	٥٢.١	٥٥.٩
	١٩ February	٠.٠٠	٠.٠٠	٢٢.٩	٢٣.٨	٦٠.٨	٦٢.٧
	٣ February	٠.٠٠	٠.٠٠	٢١.١	٢٣.٥	٦٣.٧	٦٣.٨
	١٥ Mars	٠.٠٠	٠.٠٠	٢٥.٨	٢٦.٤	٦٥.٩	٦٦.١
spring	٢٧ Mars	٠.٠٠٤	٠.٠٠٦	٢٥.٧	٢٥.١	٦٨.٨	٦٧.٣
	٨ April	٦.١٩	٦.٠٣	٣٠.٢	٢٩.٨	٦٦.٧	٦٨.٢
	٢٠ April	٧.٢٥	٧.١٩	٣٢.٤	٣١.٩	٦٩.٦	٧٠.٣
	٢ May	١.٩٩	١.٩	٢٤.٨	٣٠.٨	٦٢.١	٦٣.٨
	١٤ May	٣.١٥	٣.٠٧	٢٨.٣	٢٨.٥	٦٣.٣	٦٣.٨
	٢٦ May	٥.٦٨	٥.٥١	٢٩.٦	٢٩.٣	٦٨.٥	٦٩.١
	٧ June	٥.٩٢	٥.٦٤	٣١.٤	٣٣.٥	٦٨.٢	٦٩.٢
	١٩ June	٥.٥١	٥.٣	٣٣.٥	٣٢.٧	٦٧.٨	٦٦.٧
summer	١ July	٣.٥١	٣.٢١	٣٤.٢	٣٤.٩	٦٥.٧	٦٦.١
	١٣ July	٢.٣١	٢.١٢	٣٤.٧	٣٥.١	٦٣.٥	٦٤.٢
	٢٥ July	٢.٢١	١.٨٣	٣٣.٧	٣٤.٨	٦٠.٢	٦٠.٤
	٦ August	٤.٥٢	٤.٢٢	٣١.٤	٢٩.٨	٦٥.٧	٦٥.٣
	١٨ August	٤.٩٦	٤.٣٥	٢٩.٩	٣٣.٧	٦٨.٩	٦٨.٢
	٣٠ August	٥.٤٧	٥.٠٨	٣٠.٧	٣٥.٦	٦٦.٦	٦٦.٩
Correlation coefficient ٢٠٠٩/ ٢٠١٠ (%Mean Infection/ Mean daily Temperature)							٠.٧١
Correlation coefficient ٢٠١٠/ ٢٠١١ (%Mean Infection/ Mean daily Temperature)							٠.٦٦
Correlation coefficient ٢٠٠٩/ ٢٠١٠ (%Mean Infection/ Mean daily relative humidity)							٠.٢٩
Correlation coefficient ٢٠١٠/ ٢٠١١ (%Mean Infection/ Mean daily relative humidity)							٠.٣٣
Regression coefficient ٢٠٠٩/ ٢٠١٠ (%Mean Infection/ Mean daily Temperature)							٠.٢٩
Regression coefficient ٢٠١٠/ ٢٠١١ (%Mean Infection/ Mean daily Temperature)							٠.٢٥
Regression coefficient ٢٠٠٩/ ٢٠١٠ (%Mean Infection/ Mean daily relative humidity)							٠.١٣
Regression coefficient ٢٠١٠/ ٢٠١١ (%Mean Infection/ Mean daily relative humidity)							٠.١٧

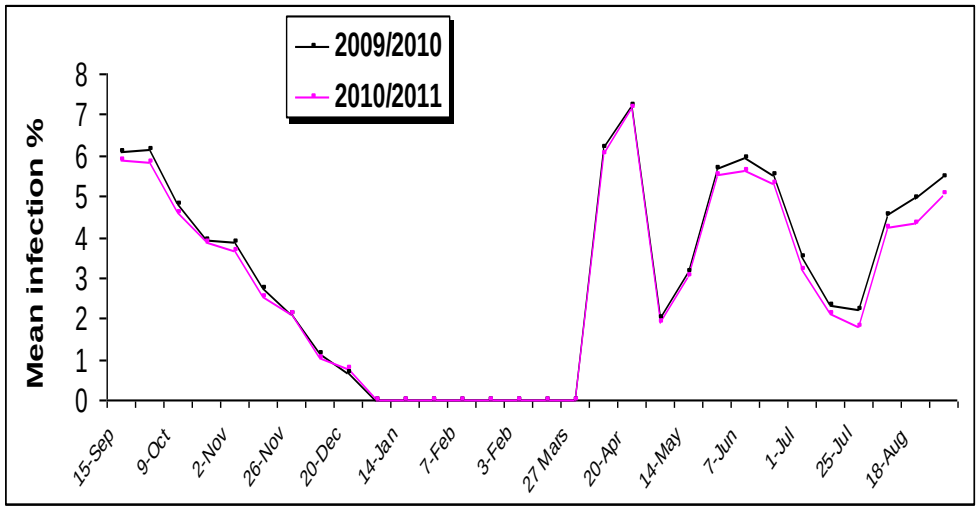

Fig ١ : Development the infection of honeybee brood by chalkbrood disease during ٢٠٠٩ / ٢٠١٠ and ٢٠١٠/٢٠١١ seasons at Minia region.

Determination of regression coefficient at first season (٢٠٠٩/٢٠١٠) between percentage of infection and both temperature and relative humidity revealed that there were positive coefficients (٠.٢٩, ٠.١٣ respectively), which means by increase temperature by one degree, infection percentage increase about ٠.٢٩٪ and also, increasing relative humidity by one percentage will correspond by increasing infection percentage by ٠.١٣٪. Regression coefficient at the second season (٢٠١٠/٢٠١١) between percentage of infection and both temperature and relative humidity was ٠.٢٥, ٠.١٧, respectively, which means by increasing temperature about one degree or relative humidity by ١٪, infection percentage increase about ٠.٢٥٪ and ٠.١٧٪, respectively.

These results are in agreement with the obtained data by Sommaruga, (١٩٨٣) who found that mean infection percentage of chalkbrood was up to ٨.٣٪ in autumn. Also, Debeljak *et al.*, (١٩٩١) reported that the infection level was up to ٤.٦٪ brood deaths by chalkbrood. In addition, Puerta *et al*., (١٩٩٤) found that chalkbrood

disease occurs suddenly in the latter part of winter, spring and finally in late summer. Mossadegh and Alizadeh, (١٩٩٥) inspected ١٤ provinces of Iran, colonies of *A.mellifera* for chalkbrood disease, which was present in ٦ of the provinces, with an overall infection rate of ٨٪. Hornitzky, (٢٠٠١) and Simsek, (٢٠٠٥) reported that chalkbrood infection percentage up to ٧.٥٦٪,in Tasmania, Western Australia and the Northern Territory. Gilliam *et al.* (١٩٩٧) who found that chalkbrood infection percentage was up to ٢٠٪ in spring and reduced to ٣٪ in summer and autumn, in winter infection was less ١٪. Flores *et al.,* (١٩٩٦) found that when kept larvae were at ٢٥ degrees and ٦٨٪ RH for ٦ days after sealing, mummification occurred in ٩٥٪ of larvae. Without the initial period of cooling, mummification was ٧٨٪ and it was even lower when sealed larvae were kept at ٣٠ degrees (١٥.٣٪) or ٣٥ degrees (٢.٢٪).

٢. Controlling chalkbrood diseases by treatment with an antibiotic (Terramycine) and other three chemicals:-

Using Terramycine as an antibiotic and other three chemicals were applied over the year except that period of nectar flow.

Monitoring the infection with chalkbrood during autumn:

Data in Table (٢) and Figure (٢) showed the efficiency of the tested chemicals used to control chalkbrood in autumn of the first year (٢٠٠٩). Of the four tested chemicals, acetyl salicylic acid gave the highest reduction percentages (٥٢.٥٤, ٥٢.٠٣ and ٥١.٢٨%) of chalkbrood infection when a mixed with sugar powder, candy and sugar syrup, respectively. Thymol efficacy occupied the second rank (٣٦.١٦,٣٦.١٥ and ٣٣.٣٩٪, respectively), then Terramycine (٣٣.٢٦, ٣٣.٢٦and ٢٨.٩٦ %, respectively). While potassium sorbate occupied the last position (٢.٢٥, ٢.١٣ and ٣.٣٠٪) for the same carriers mentioned above, respectively.

On the other hand using sugar powder as a carrier of the tested chemicals seem to be support the efficacy of these chemicals where the lowest percentages of infection were recorded (١.٥٩,٢.١٠,٢.٢٥ and ٣.٢٨٪) with acetyl salicylic acid, Thymol, Terramycine and potassium

sorbate, respectively. However, using sugar syrup as carrier resulted in the lowest efficacy of these chemicals were represented in increasing infection percentages by ١.٧١, ٢.٣٤, ٢.٥٠ and ٣.٤٠ with acetyl salicylic acid, Thymol, Terramycine and potassium sorbate, respectively.

Table ٢: Mean percentages of chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in autumn, ٢٠٠٩ at Minia region.

Treatments / Date	Acetyl salicylic acid			Thymol			Terramycine			Potassium sorbate			Control			*L.S.R at ٥٪
	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	
Before treatment	٥.٧٦	٥.٨٣	٥.٦٧	٥.٧٢	٥.٨٠	٥.٦٨	٥.٨٦	٥.٨٢	٥.٧٠	٥.٨٠	٥.٧٣	٥.٧٩	٥.٨٤	٥.٦٨	٥.٦٦	
١٥ September	٢.٦٩ n	٢.٧٣ mn	٢.٨٨ lmn	٣.٥٣ k	٣.٧٨ j	٣.٨٣ ij	٤.٢٤ h	٤.٣١ gh	٤.٥٦ f	٥.٦١ e	٥.٦٨ de	٥.٧٢ cde	٥.٨٢ b-e	٥.٩٦ ab	٦.٠٩ a	٠.٢٢٤
٢٧ September	٣.٢٤ n	٣.٢٩ mn	٣.٣٣ lmn	٣.٩٥ ij	٣.٧٤ k	٣.٩٤ j	٤.٤٠ h	٤.٤١ gh	٤.٧٢ f	٥.٧٢ e	٥.٨٢ de	٥.٨٨ cd	٥.٨٩ bcd	٦.٠٧ a	٦.١٣ a	٠.١١٨
٩ October	٢.٨٨ l	٢.٩١ kl	٢.٩٦ jkl	٣.١٢ i	٣.٣١ gh	٣.٣١ h	٣.٣٢ fgh	٣.٥١ e	٣.٧٢ D	٤.٧١ ab	٤.٧٥ ab	٤.٧٨ a	٤.٦٣ bc	٤.٥٣ c	٤.٨١ a	٠.١٣١
٢١ October	٢.١١ n	٢.١٨ mn	٢.٢١ lm	٢.٨٩ h	٣.١٢ g	٣.٣٩ f	٢.٥٣ k	٢.٦٢ j	٢.٧٧ I	٣.٦٣ e	٣.٧٢ d	٣.٨١ c	٣.٨٢ bc	٣.٨٥ abc	٣.٩٢ a	٠.٠٧٥
٢ November	١.٩٢ o	٢.٢١ n	٢.٣١ m	٢.٦١ l	٢.٦٦ kl	٣.٠١ j	٣.٢٥ i	٣.٣٢ h	٣.٣٦ gh	٣.٧١ e	٣.٧٣ cde	٣.٨٢ b	٣.٥٩ f	٣.٧٢ de	٣.٨٩ a	٠.٠٥٣
١٤ November	٠.٦٧ m	٠.٦٩ klm	٠.٦٨ lm	١.٢٣ gh	١.٢٦ e-h	١.٢٣ h	١.٠٣ j	١.١٥ i	١.٢٦ fgh	٢.٦٢ cd	٢.٦٥ bc	٢.٦٨ ab	٢.٧٣ a	٢.٥٨ d	٢.٧١ a	٠.٠٥٥
٢٦ November	٠.٦٢ j	٠.٦٦ ij	٠.٧٢ hij	١.١٩ d	١.٢٠ cd	١.٢١ bcd	٠.٩٩ g	١.٠٤ fg	١.٠٦ efg	٢.٠٧ a	٢.٠٨ a	٢.١٢ a	٢.٠٢ a	٢.١٠ a	٢.١٣ a	٠.١٣٠
٨ December	٠.١٥ j	٠.١٩ ij	٠.٢١ g-j	٠.٣١ e-j	٠.٦٩ de	٠.٩٤ bc	٠.٢١ hig	٠.٢٣ f-j	٠.٧٨ C	١.٠٦ ab	١.٠٩ ab	١.١٢ a	١.٠٣ ab	١.٠٧ ab	١.١٥ a	٠.١٧٥
٢٠ December	٠.٠٢ l	٠.٠٩ ijk	٠.٠٧ jkl	٠.٠٤ kl	٠.١٤ k	٠.١٩ ghi	٠.٢٤ def	٠.٢٨ hi	٠.٣٠ fgh	٠.٤٢ c	٠.٥٦ b	٠.٦٣ a	٠.٦٥ a	٠.٦٤ a	٠.٦٦ a	٠.٠٥٠
Mean	١.٥٩	١.٦٦	١.٧١	٢.١٠	٢.٢١	٢.٣٤	٢.٢٥	٢.٣٢	٢.٥٠	٣.٢٨	٣.٣٤	٣.٤٠	٣.٣٥	٣.٣٩	٣.٥٠	
Reduction%	٥٢.٥٤ a	٥٢.٠٣ b	٥١.٢٨ c	٣٦.١٦ de	٣٦.١٥ e	٣٣.٣٩ fgh	٣٣.٢٦ gh	٣٣.٢٦ h	٢٨.٩٦ I	٢.٢٥ kl	٢.١٣ l	٣.٣٠ j	٠.٣٢٦			

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)
*L.S.R. Least significant range (Duncan range at ٥٪).

Time appearance and control of chalkbrood disease

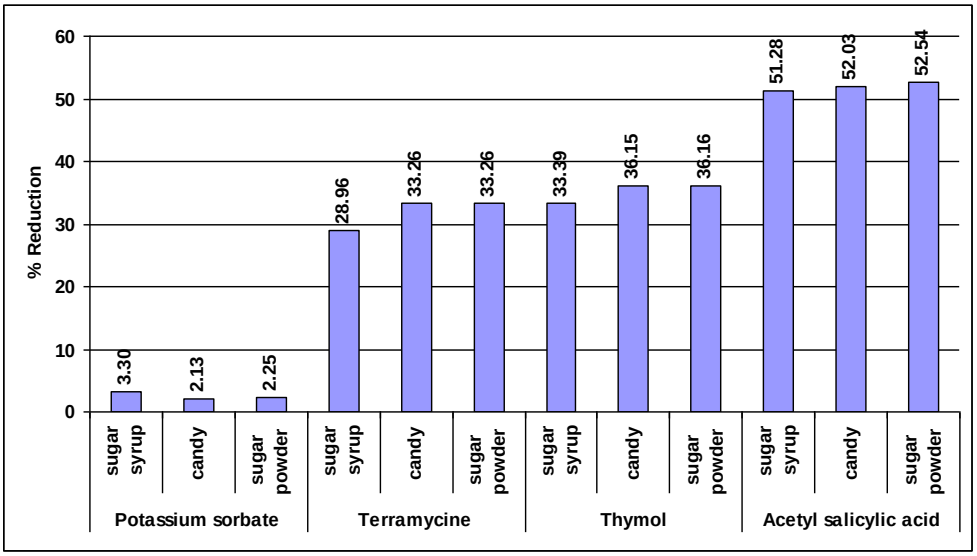

Fig ٢: Mean percentages of reduction in chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in autumn, ٢٠٠٩ at Minia region.

Statistical analysis of the obtained data showed that there were significant differences among the efficacy of different treatments for explanation acetyl salicylic acid was statically the most effective treatment. In contrast, potassium sorbate was inferior.

Data presented in Table (٣) and Figure (٣) referred to the efficiency of the chemicals used to control chalkbrood for the second year (٢٠١٠). Data indicated that the mean efficiency of the tested chemicals could be arranged in the following descending order; acetyl salicylic acid > Thymol > Terramycine > potassium sorbate which resulted in (٥١.٨١,٥ ٠٢.٧ and ٥٠.٣١٪), (٣٦.٩١, ٣٦.٦٧ and ٣٦.٥١٪), (٣١.٣١,٣ ٠.٩٦ and ٣٠.٧٩٪) and (١.٩١,١.٢٥ and ١.٣٩٪) reduction percentages of disease infection when used with sugar powder, candy and sugar syrup, respectively.

Table ٣: Mean percentages of chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in autumn, ٢٠١٠ at Minia region.

Treatments / Date	Acetyl salicylic acid			Thymol			Terramycine			Potassium sorbate			Control			*L.S.R at ٥٪
	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	
Before treatment	٥.٧٧	٥.٧٥	٥.٧٠	٥.٨٤	٥.٨٣	٥.٧٥	٥.٨٩	٥.٨٧	٥.٦٥	٥.٧٢	٥.٨٢	٥.٧٠	٥.٧٠	٥.٨٣	٥.٧٠	
١٥ September	٢.٥٥ n	٢.٦٣ mn	٢.٧٠ lm	٣.٢٢ k	٣.٣٤ j	٣.٦٢ I	٣.٨٦ h	٣.٩٥ gh	٤.٠٥ f	٥.٣١ e	٥.٦٢ c	٥.٨٥ a	٥.٣٩ de	٥.٦٨ bc	٥.٨٩ a	٠.٠٩٢
٢٧ September	٣.١٥ l	٣.٢٢ kl	٣.٢٨ jkl	٣.٧٥ i	٣.٨٢ gh	٣.٩٣ H	٤.٠٩ d-j	٤.٢٦ c-j	٤.٣٥ b-i	٥.١١ a-d	٥.٥٩ a	٥.٧٦ a	٥.٢١ a-d	٥.٦٣ a	٥.٨٢ a	١.١٧٧
٩ October	٢.٥٦ o	٢.٦٨ n	٢.٧٦ m	٣.١١ l	٣.٢٤ k	٣.٣٤ j	٣.٤٦ i	٣.٤٨ h	٣.٥٢ g	٤.١٩ f	٤.٣٣ d	٤.٥٤ b	٤.٢٣ e	٤.٣٦ c	٤.٥٨ a	٠.٠٠١
٢١ October	٢.٠٩ o	٢.١٣ n	٢.٢٥ m	٢.٦٢ hi	٢.٦٠ j	٢.٨٢ G	٢.٤٢ l	٢.٤٨ k	٢.٦٢ i	٣.٦٦ f	٣.٧٢ e	٣.٨٢ b	٣.٧٢ de	٣.٨٠ c	٣.٨٥ a	٠.٠٠٢
٢ November	١.٧٢ o	١.٩٦ n	٢.١٠ m	٢.٢٣ l	٢.٣٢ k	٢.٤٦ j	٢.٨٩ i	٢.٩٩ h	٣.٠٩ g	٣.٢٩ f	٣.٣٧ d	٣.٥٨ b	٣.٣٢ e	٣.٤٥ c	٣.٦٧ a	٠.٠٠٩
١٤ November	٠.٦٥ n	٠.٧٨ m	٠.٨٩ l	١.١٣ k	١.٢٠ j	١.٢٢ ij	١.٢٤ hig	١.٣١ g	١.٣٥ fg	٢.٣٠ e	٢.٤٢ c	٢.٥٠ a	٢.٣٥ de	٢.٤٤ bc	٢.٥٣ a	٠.٠٥٣
٢٦ November	٠.٥٥ o	٠.٦٣ n	٠.٨٠ m	١.١٠ l	١.١٦ j	١.٢٠ H	١.١٢ k	١.١٩ i	١.٢٣ g	١.٧٩ f	١.٩٣ d	٢.٠٨ b	١.٨٢ e	١.٩٧ c	٢.١١ a	٠.٠٠١
٨ December	٠.١١ o	٠.١٣ n	٠.١٦ m	٠.٣٢ i	٠.٣٦ h	٠.٤٤ G	٠.٢٣ l	٠.٢٦ k	٠.٣١ j	٠.٨٥ f	٠.٨٩ d	٠.٩٦ b	٠.٨٦ e	٠.٩٢ c	١.٠٣ a	٠.٠٠٢
٢٠ December	٠.٠٠ o	٠.٠٣ n	٠.٠٨ m	٠.٢٥ i	٠.٢٨ h	٠.٣٣ G	٠.١٦ l	٠.١٩ k	٠.٢٢ j	٠.٥٠ f	٠.٦٥ d	٠.٧٢ b	٠.٥٣ e	٠.٦٨ c	٠.٧٥ a	٠.٠٠٢
Mean	١.٤٩	١.٥٨	١.٦٧	١.٩٧	٢.٠٤	٢.١٥	٢.١٦	٢.٢٣	٢.٣٠	٣.٠٠	٣.١٧	٣.٣١	٣.٠٥	٣.٢١	٣.٣٦	
Reduction%	٥١.٨١ a	٥٠.٢٧ a	٥٠.٣١ a	٣٦.٩١ bcd	٣٦.٦٧ cd	٣٦.٥١ D	٣١.٣١ efg	٣٠.٩٦ fg	٣٠.٧٩ g	١.٩١ hij	١.٢٥ j	١.٣٩ ij	٤.٣٦٨			

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)

*L.S.R. Least significant range (Duncan range at ٥٪).

Time appearance and control of chalkbrood disease

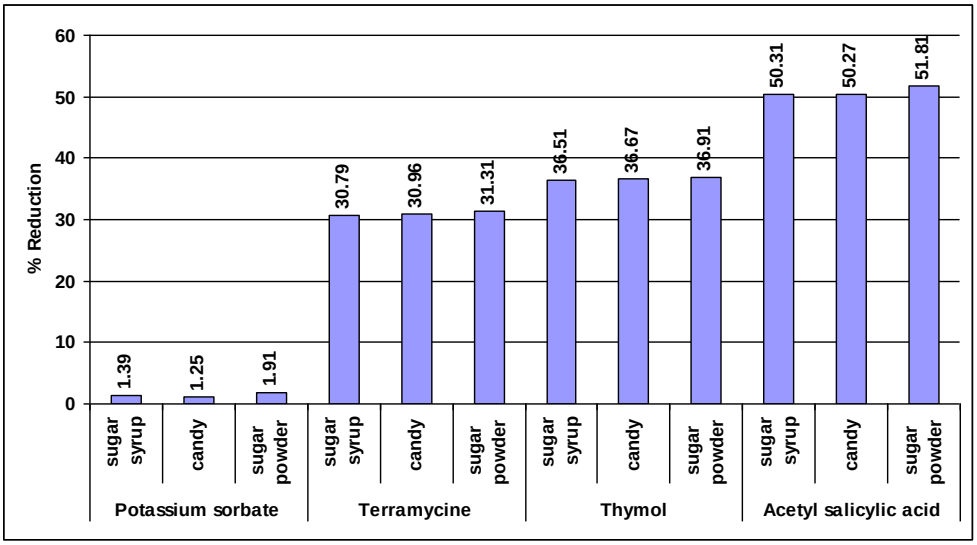

Fig ٣: Mean percentages of reduction in chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in autumn, ٢٠١٠ at Minia region.

The present results reconfirmed that acetyl salicylic acid was the most effective on chalkbrood disease than other tested chemicals, and sugar powder was more suitable for carrying these chemicals than other tested carriers. Suitability of sugar powder may be attributed to two possible reasons, first of them, its dryness nature which can keep the action of mixed chemicals in a good stat for along time. While the second reason may be attributed to absence of humidity, which encourage formation of chalkbrood spors, that humidity is available in case of sugar syrup and to the same extend in candy.

The data expressed as % reduction in chalkbrood infection were statistically analyzed showing significant differences among chemical treatments. However, the differences between carriers were not significant (Table ٣).

These results are in agreement with Menapace and Hale, (١٩٨١) who found that potassium sorbate did not prevent or control chalkbrood under field conditions. Jenko *et al.*, (١٩٩١) who found that

using fungicide such as acetyl salicylic acid in off-season recorded ٨٦٪ of colonies had less chalkbrood mummies than untreated controls. Davis and Ward, (٢٠٠٣) who found that Thymol is a major component of thyme oil, is highly active against fungal brood diseases and using Thymol in full season (winter and summer) resulted in ٣٩ % reduction. Also, they found that salicylic acid at ١٠٠ ppm was effective against fungal brood diseases. Flores *et al.,* (٢٠٠٤) who found that antibiotics like oxytetracycline reduced infection percentage up to ٤٢٪ of chalkbrood in the honeybee (*Apis mellifera* L.). They concluded that it would be of great interest to verify the same effect in the long term in apiaries. Ali, (٢٠٠٧) found that Thymol completely inhibited the growth of the fungus of *Aspergillus flavus*. Harz *et al.,* (٢٠٠٨) found that using acetyl salicylic acid effectively control the microorganisms associated with the honeybee colonies

Chemical control of chalkbrood disease during winter

Results in Table (٤) and Figure (٤) of the first year of study (winter, ٢٠١٠) showed that the efficacy of acetyl salicylic acid surpassed the efficacy of Terramycine which in turn surpassed the efficacy of Thymol, while the efficacy of potassium sorbate came in last position.

When candy was used as a carrier, reduction percentages of chalkbrood infection at the end of winter season were ٨٠.٤١, ٦٠.٩٦, ٦٠.٨٣ and ٠.٧٠٪ for acetyl salicylic acid, Terramycine, Thymol and potassium sorbate, respectively. However using of the tested chemicals with sugar powder or sugar syrup resulted in less reduction percentages of the disease infection (Table ٧).

Time appearance and control of chalkbrood disease

Table ٤: Mean percentages of chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in winter, ٢٠١٠ at Minia region.

Treatments / Date	Acetyl salicylic acid			Thymol			Terramycine			Potassium sorbate			Control			*L.S.R at ٥٪
	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar Syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	
Before treatment	٥.٨٣	٥.٨٠	٥.٦٧	٥.٧٢	٥.٨٠	٥.٦٨	٥.٨٦	٥.٨٢	٥.٧٠	٥.٨٥	٥.٧٢	٥.٦٨	٥.٨٤	٥.٦٨	٥.٦٦	
٢ January	٠.٠٠١ l-o	٠.٠٠١ mno	٠.٠٠٢ g-k	٠.٠٠٢ hk	٠.٠٠٢ ijk	٠.٠٠٢ jk	٠.٠٠١ no	٠.٠٠٢ k	٠.٠٠١ o	٠.٠٠٣ c-f	٠.٠٠٣ def	٠.٠٠٤ b	٠.٠٠٣ bf	٠.٠٠٣ f	٠.٠٠٥ a	٠.٠٠٢
١٤ January	٠.٠٠٠ h-o	٠.٠٠٠ i-o	٠.٠٠٠ j-o	٠.٠٠١ efg	٠.٠٠٠ k-o	٠.٠٠٠ l-o	٠.٠٠٠ mno	٠.٠٠٠ no	٠.٠٠٠ o	٠.٠٠١ fg	٠.٠٠٢ bcd	٠.٠٠١ g	٠.٠٠٢ cd	٠.٠٠٢ d	٠.٠٠٣ a	٠.٠٠١
٢٦ January	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
٧ February	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
١٩ February	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
٣ March	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
١٥ March	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
Mean	٠.٠٠٠١	٠.٠٠٠ ١	٠.٠٠١	٠.٠٠١	٠.٠٠١	٠.٠٠١	٠.٠٠٠ ١	٠.٠٠١	٠.٠٠٠ ١	٠.٠٠ ١	٠.٠٠٢	٠.٠٠ ٢	٠.٠٠٢	٠.٠٠ ٢	٠.٠٠٣	
Reduction%	٧٩.٩٧ d	٨٠.٤١ bcd	٧٥.٠٤ f	٣٨.٧٤ i	٦٠.٨٣ h	٧٥.٠٩ ef	٨٠.٠٧ cd	٦٠.٩٦ gh	٨٧.٥٩ a	٢٠.١ ٤ k	٠.٧٠ l	٣٧.٧ ٢ j	٠.٧٧٠			

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)
*L.S.R. Least significant range (Duncan range at ٥٪).

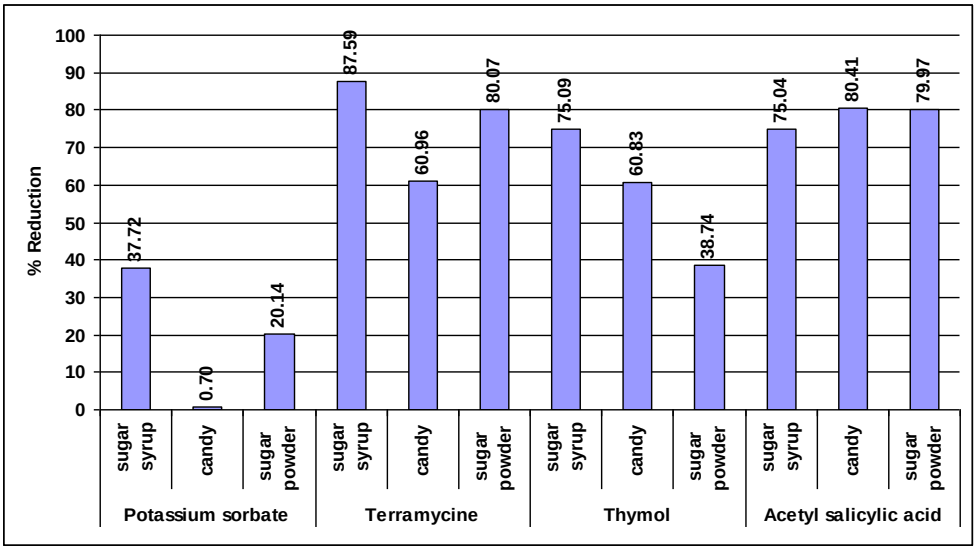

Fig ٤: Mean percentages of reduction in chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in winter, ٢٠١٠ at Minia region.

In the second season (winter ٢٠١١), data in Table (٥) and Figure (٥) showed that using acetyl salicylic acid with sugar powder, candy and sugar syrup resulted in the lowest mean infection percentages of chalkbrood infection (١.٤٩,١.٥٨ and ١.٦٧٪), followed by Thymol (١.٩٧, ٢.٠٤ and ٢.١٥٪), then Terramycine (٢.١٦,٢.٢٣ and ٢.٣٠٪). While using potassium sorbate recorded the highest mean infection percentages (٣.٠٠, ٣.١٧ and ٣.٣١٪) for the same carriers mentioned above, respectively. According to reduction percentages of the disease infection at the end of winter season, the efficiency of the tested materials could be arranged in the following descending order; acetyl salicylic acid > Thymol > Terramycine > potassium sorbate which resulted in reducing chalkbrood infection by (٥١.٨١, ٥٠.٣١ and ٥٠.٢٧٪), (٣٦.٩١, ٣٦.٦٧ and ٣٦.٥١٪), (٣١.٣١, ٣٠.٩٦ and ٣٠.٧٩٪) and (١.٩١, ١.٣٩ and ١.٢٥٪) when they were carried on sugar powder, sugar syrup and candy, respectively.

Treatments	Acetyl salicylic acid	Thymol	Terramycine	Potassium sorbate	Control	*L.S.R at ٥٪

Time appearance and control of chalkbrood disease

	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	
Before treatment	٥.٧٧	٥.٧٥	٥.٧٠	٥.٨٤	٥.٨٣	٥.٧٥	٥.٨٩	٥.٨٧	٥.٦٥	٥.٧٢	٥.٨٢	٥.٧٠	٥.٧٠	٥.٨٣	٥.٧٠	
٢ January	٠.٠٠٠ h-o	٠.٠٠٢ i-o	٠.٠٠٣ j-o	٠.٠٠١ f	٠.٠٠٢ k-o	٠.٠٠٣ l-o	٠.٠٠٢ mno	٠.٠٠٢ no	٠.٠٠١ o	٠.٠٠٣ g	٠.٠٠٤ ef	٠.٠٠٥ c	٠.٠٠٣ f	٠.٠٠٤ bc	٠.٠٠٦ a	٠.٠٠٢
١٤ January	٠.٠٠٠ h-o	٠.٠٠٠ i-o	٠.٠٠٠ j-o	٠.٠٠٢ f	٠.٠٠٠ k-o	٠.٠٠٠ l-o	٠.٠٠٠ mno	٠.٠٠٠ no	٠.٠٠٠ o	٠.٠٠١ g	٠.٠٠٢ ef	٠.٠٠٣ c	٠.٠٠٢ f	٠.٠٠٣ bc	٠.٠٠٣ abc	٠.٠٠١
٢٦ January	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
٧ February	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
١٩ February	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
٣ March	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
١٥ March	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	٠.٠٠٠	—
Mean	٠.٠٠٠	٠.٠٠١	٠.٠٠١	٠.٠٠١	٠.٠٠١	٠.٠٠١	٠.٠٠١	٠.٠٠١	٠.٠٠٠١	٠.٠٠١	٠.٠٠٢	٠.٠٠٣	٠.٠٠٢	٠.٠٠٢	٠.٠٠٣	
Reduction%	١٠٠.٠٠	٧١.٠٣	٦٦.٦٧	٤١.٤٤	٧١.٤٣	٦٦.٩٦	٦١.٢٩	٧١.٦٢	٨٨.٧٩	٢٠.٢٨	١٤.١٤	١١.١١	٠.٠٩٣			

Table ٥: Mean percentages of chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in winter, ٢٠١١ at Minia region.

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)
*L.S.R. Least significant range (Duncan range at ٥٪).

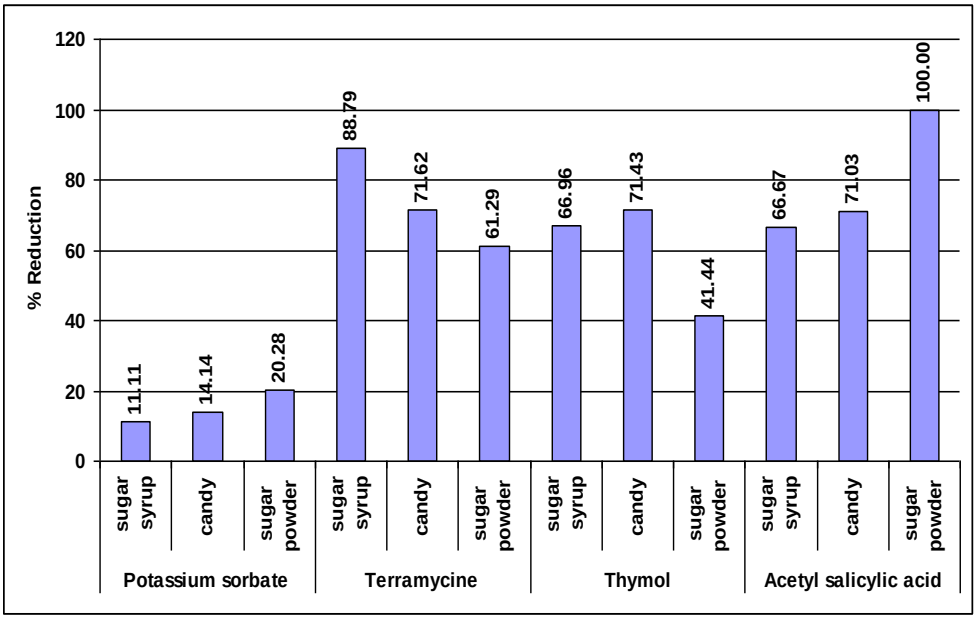

Fig ٥: Mean percentages of reduction in chalkbrood infection when honeybee colonies were treated with an antibiotic (Terramycine) and other three chemicals in winter, ٢٠١١ at Minia region.

Statistical analysis of the obtained data showed that there was significant difference between efficacy of tested chemicals and potassium sorbate was the least effective treatment.

In the present study acetyl salicylic acid was the greatest effective treatment. These results are in agreement with Jenko *et al.*, (١٩٩١) who found that using acetyl salicylic acid in winter resulted in ٨٢٪ reduction percentage. Our results with thymol were confirmed by Ali, (٢٠٠٧) who found that Thymol inhibited completely the growth of the fungus of *Aspergillus flavus*. Harz *et al.*, (٢٠٠٨) found that acetyl salicylic acid successfully control microorganisms which affect of honeybee colonies.

However, the present results are far from those results obtained by Mourad *et al.*, (٢٠٠٥) who found that using Thymol recorded ٠.٥٪ reduction in mummies numbers resulted by fungal diseases.

Time appearance and control of chalkbrood disease

Monitoring chalkbrood infection during spring

Results in Table (٦) and Figure (٦) showed the efficiency of chemicals that used to control chalkbrood have a positive in spring of the first year (٢٠١٠) which could be arranged in the following descending order; potassium sorbate (٤.٢٨, ٤.٢٢ and ٤.١٣٪) > Thymol (٢.٤٠, ٢.٣٥ and ٢.٢٤٪) > Terramycine (٢.١٩, ٢.١٣ and ٢.٠٢٪) > acetyl salicylic acid (١.٧٨, ١.٧٢ and ١.٦٣٪) when they used with sugar syrup, candy and sugar powder, respectively.

Table ٦: Mean percentages of chalkbrood infection monitored during spring of ٢٠١٠ in honeybee colonies previously treated in winter ٢٠٠٩/٢٠١٠ with the four tested chemicals at Minia region.

Treatments / Date	Acetyl salicylic acid			Thymol			Terramycine			Potassium sorbate			Control			L.S.R at ٥٪
	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	
٢٧ March	٠.٠٠ k-o	٠.٠٠ l-o	٠.٠٠ mno	٠.٠٠١ hij	٠.٠٠١ ij	٠.٠٠٢ d-g	٠.٠٠ no	٠.٠٠ o	٠.٠٠١ j	٠.٠٠٢ efg	٠.٠٠٢ fg	٠.٠٠٤ ab	٠.٠٠٢ g	٠.٠٠٣ c	٠.٠٠٤ b	٠.٠٠ ١
٨ April	٢.١١ j	٢.١٥ ij	٢.١٩ hij	٢.٢٤ g-j	٢.٣١ f-j	٢.٣٤ e-j	٢.٥٣ d-j	٢.٦٢ c-j	٢.٦٦ b-j	٥.٢٢ a-j	٥.٥٢ a-d	٥.٤٥ a-e	٦.٠٧ a	٦.١١ a	٦.١٩ a	٣.١٣ ٢
٢٠ April	٢.٢٩ m	٢.٣١ lm	٢.٣٥ klm	٣.٠٧ g	٣.١١ fg	٣.١٩ efg	٢.٧٨ j	٢.٨٢ ij	٢.٩١ hij	٦.٨٩ d	٦.٩٥ cd	٦.٩٨ bcd	٧.١١ ab	٧.١٧ a	٧.٢٥ a	٠.١٥ ٠
٢ May	٠.٦٢ m	٠.٦٩ l	٠.٧٣ lm	٠.٨٤ d-j	٠.٩٧ c-j	٠.٩٩ b-j	٠.٩٠ j	٠.٩٢ ij	٠.٩٤ d-i	١.٧٤ a	١.٨٤ a	١.٩٠ a	١.٩٢ a	١.٩٥ a	١.٩٩ a	٠.٦٧ ٣
١٤ May	١.٥٥ m	١.٦٢ lm	١.٧٣ k	٢.٣١ j	٢.٥٣ ghi	٢.٥١ hi	٢.٤٨ i	٢.٥٥ f-i	٢.٧١ e	٣.٠٠ cd	٢.٩٩ d	٣.١١ ab	٣.٠٣ bcd	٣.١٢ ab	٣.١٥ a	٠.١٠ ٦
٢٦ May	١.٩٠ m	١.٩٥ lm	٢.٠٩ k	٣.١٤ g	٣.٢٥ f	٣.٣٢ ef	٢.٦٦ j	٢.٨٥ i	٢.٩٦ i	٥.٣١ d	٥.٥٠ c	٥.٥٤ bc	٥.٦٢ a	٥.٦٥ a	٥.٦٨ a	٠.٠٧ ٥
٧ June	٢.٣٣ j	٢.٥١ ij	٢.٧١ g-j	٣.١٢ d-g	٣.٣٣ c-f	٣.٥٣ bcd	٢.٥٥ hij	٢.٩٩ fg	٣.٠٠ efg	٥.٦٤ a	٥.٦٢ a	٥.٧١ a	٥.٨٧ a	٥.٨٩ a	٥.٩٢ a	٠.٤٣ ٣
١٩ June	٢.٢٢ n	٢.٥٤ i	٢.٤٢ j	٣.٢١ h	٣.٣٣ g	٣.٣٤ fg	٢.٢٣ mn	٢.٢٩ l	٢.٣٢ kl	٥.٢٦ e	٥.٣٦ d	٥.٥٤ a	٥.٤٥ c	٥.٤٧ bc	٥.٥١ ab	٠.٠٥ ٣
Mean	١.٦٣	١.٧٢	١.٧٨	٢.٢٤	٢.٣٥	٢.٤٠	٢.٠٢	٢.١٣	٢.١٩	٤.١٣	٤.٢٢	٤.٢٨	٤.٣٨	٤.٤٢	٤.٤٦	

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)
*L.S.R. Least significant range (Duncan range at ٥٪).

Results also, recorded that using sugar powder as carrier enhanced the activity of tested chemicals that resulted in the lowest infection percentages of chalkbrood (٤.١٣, ٢.٠٢, ٢.٢٤ and ١.٦٣٪) for potassium sorbate, Terramycine, Thymol and acetyl salicylic acid, respectively. In contrast using sugar syrup recorded the highest mean infection percentages (٤.٢٨, ٢.٤٠, ٢.١٩ and ١.٧٨٪) while using candy

as carrier of these chemicals resulted in decreasing of the disease infection by figures came in between the two levels mentioned above.

Statistical analysis of the obtained data showed that there were significant differences among the efficacy of different treatments and potassium sorbate gave unsatisfactory results in this respect.

In spring of ٢٠١١, data in Table (٧) and Figure (٧) showed that the highest infection percentages were recorded for those colonies that were treated with potassium sorbate (٤.٢٨, ٤.١٨ and ٤.٠٥٪), while the lowest infection percentages were ١.٦٩, ١.٦١ and ١.٤٧٪ in acetyl salicylic acid treatments with sugar syrup, candy and sugar powder, respectively.

Table ٧: Mean percentages of chalkbrood infection monitored during spring of ٢٠١١ in honeybee colonies previously treated in winter ٢٠١٠/٢٠١١ with the four tested chemicals at Minia region.

Treatments / Date	Acetyl salicylic acid			Thymol			Terramycine			Potassium sorbate			Control			*L.S.R at ٥٪
	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	
٢٧ March	٠.٠٠ mno	٠.٠٠ no	٠.٠٠١ jkl	٠.٠٠٢ hi	٠.٠٠٢ i	٠.٠٠٣ g	٠.٠٠ o	٠.٠٠١ kl	٠.٠٠١ l	٠.٠٠٤ c-f	٠.٠٠٤ def	٠.٠٠٦ ab	٠.٠٠٤ ef	٠.٠٠٤ f	٠.٠٠٦ b	٠.٠٠١
٨ April	٢.٠١ o	٢.٠٩ n	٢.١٢ mn	٢.٣٥ l	٢.٣٩ kl	٢.٤١ jk	٢.٥٥ i	٢.٦٠ hi	٢.٦٨ g	٥.٧٢ f	٥.٩٠ d	٥.٩٦ bc	٥.٧٨ ef	٥.٩٢ cd	٦.٠٣ a	٠.٠٥٣
٢٠ April	٢.٢٢ j	٢.٢٩ ij	٢.٣٢ hij	٢.٨٩ d-g	٢.٩٦ c-g	٣.٠٨ b-g	٢.٦٥ g-j	٢.٧٢ f-i	٢.٧٧ efg	٦.٨٢ a	٧.٠٠ a	٧.٠٥ a	٦.٨٥ a	٧.٠٨ a	٧.١٩ a	٠.٤٣٦
٢ May	٠.٥٣ m	٠.٦٠ l	٠.٧١ k	٠.٨٦ j	٠.٩٠ hij	٠.٨٩ ij	٠.٩٣ ghi	٠.٩٩ f	١.٠٥ e	١.٦٩ d	١.٨٤ b	١.٨٨ ab	١.٧٣ cd	١.٨٦ ab	١.٩٠ a	٠.٠٥٥
١٤ May	١.٢٣ o	١.٥٨ n	١.٦٠ m	١.٦٨ jk	١.٧٥ h	١.٨١ g	١.٦٨ k	١.٦٥ l	١.٧٣ i	٢.٧١ f	٢.٨٠ d	٢.٩٩ b	٢.٧٧ e	٢.٨٣ c	٣.٠٧ a	٠.٠٠٢
٢٦ May	١.٥٦ o	١.٨٠ n	١.٩٣ m	٢.٤٢ l	٢.٥١ k	٢.٨٨ j	٣.٠٩ i	٣.٢٠ h	٣.٣٥ g	٥.١٩ f	٥.٢٢ e	٥.٤٨ b	٥.٢٣ d	٥.٣٩ c	٥.٥١ a	٠.٠٠١
٧ June	٢.١٩ j	٢.٢٩ ij	٢.٥٣ hij	٢.٧٠ ghi	٢.٨٢ fgh	٢.٩١ e-h	٣.٠٢ c-g	٢.٩٢ d-h	٣.٣٣ b-e	٥.٢٠ a	٥.٤٦ a	٥.٦١ a	٥.٢١ a	٥.٥٢ a	٥.٦٤ a	٠.٤٤٣
١٩ June	٢.٠٢ l	٢.١٩ k	٢.٢٨ j	٣.٠٥ f	٣.٠٩ ef	٣.١٩ d	٢.٤٦ i	٢.٥٨ h	٢.٦٣ gh	٥.٠٩ c	٥.٢٥ a	٥.٢٨ a	٥.١١ bc	٥.٢٦ a	٥.٣٠ a	٠.٠٥٣
Mean	١.٤٧	١.٦١	١.٦٩	١.٩٩	٢.٠٥	٢.١٥	٢.٠٥	٢.٠٨	٢.١٩	٤.٠٥	٤.١٨	٤.٢٨	٤.٠٩	٤.٢٣	٤.٣٣	

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)

*L.S.R. Least significant range (Duncan range at ٥٪).

Time appearance and control of chalkbrood disease

Also, results revealed that using sugar syrup as a carrier did not give decreasing infection percentages of chalkbrood (٤.٢٨, ٢.١٩, ٢.١٥ and ١.٦٩٪ when using potassium sorbate, Terramycine, Thymol and acetyl salicylic acid, respectively). While using sugar powder as a carrier minimized infection percentages to ١.٤٧, ١.٩٩, ٢.٠٥ and ٤.٠٥٪. However using candy as a carrier resulted in medium efficacy of the tested chemicals.

These results are in agreement with Menapace and Hale, (١٩٨١) who found that potassium sorbate did not prevent or control chalkbrood under field conditions. Calderone *et al.*, (١٩٩٤) and Davis and Ward, (٢٠٠٣) who found that Thymol inhibited all growth of fungi at ١٠٠٠ ppm for ٧٢ h, Their results suggest that plant extracts might play a significant role in the management of honeybee diseases. Flores *et al.*, (٢٠٠٤) found that antibiotics like oxytetracycline recorded reduction in infection percentage up to ٤٢٪ of chalkbrood in the honeybee (*Apis mellifera* L.). They conclude that it would be of great interest to verify the same effect in the long term in apiaries.

On the other hand, the present results are in far from those results obtained by Rembold *et al.*, (١٩٨٠) who found that controlled fungal diseases by chemical compounds as oxytetracycline resulted good reducing of chalkbrood and stone brood infection up to ٧١٪. Gamber, (١٩٩٠) reported that using Thymol did not stay residuals in honey.

Monitoring chalkbrood infection during summer

Data in Table (٨) and Figure (٦) showed that using sugar powder as carrier of the tested chemicals seem to be support the efficacy of these chemicals where the lowest percentages of infection (١.١٨, ١.٦٨, ١.٦٩ and ٣.٦٦٪) were recorded with acetyl salicylic acid, Thymol, Terramycine and potassium sorbate, respectively.

However, using sugar syrup as carrier resulted in the lowest efficacy of these chemicals, which resulted in decreasing infection percentages (١.٢٨, ١.٨٧, ١.٩٣ and ٣.٨٢٪) with acetyl salicylic acid, Thymol, Terramycine and potassium sorbate, respectively.

Table ٨: Mean percentages of chalkbrood infection monitored during summer of ٢٠١٠ in honeybee colonies previously treated in winter ٢٠٠٩/٢٠١٠ with the four tested chemicals at Minia region.

Treatments / Date	Acetyl salicylic acid			Thymol			Terramycine			Potassium sorbate			Control			L.S.R at ٥٪
	Sugar powder	Candy	Sugar Syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	Sugar powder	Candy	Sugar syrup	
١ July	١.٢٢ m	١.٣١ l	١.٣٤ Kl	١.٦٢ ij	١.٦٦ j	١.٨٢ h	١.٨٤ gh	١.٨٨ fg	١.٩٢ ef	٣.٤١ d	٣.٤٩ abc	٣.٥١ a	٣.٤٤ cd	٣.٤٥ bcd	٣.٥١ a	٠.٠٥٤
١٣ July	٠.٣١ n	٠.٣٧ m	٠.٤٢ Lm	٠.٧٧ k	٠.٨١ ijk	٠.٩٣ gh	٠.٨١ jk	٠.٩٠ h	١.١١ f	٢.٠٠ e	٢.١٤ d	٢.٣١ a	٢.١٩ cd	٢.٢٥ b	٢.٣١ a	٠.٠٥٣
٢٥ July	٠.٢٢ o	٠.٢٩ n	٠.٣٤ Lm	٠.٣٤ m	٠.٦٣ j	٠.٨٧ h	٠.٥٥ k	٠.٧٦ i	٠.٩٩ g	٢.٠٥ f	٢.١٩ c	٢.٢٢ a	٢.١٣ e	٢.١٧ d	٢.٢١ b	٠.٠٠١
٦ August	١.٠٧ n	١.١١ lm	١.١٧ J	١.٨٤ h	١.٨٦ k	١.٩٤ f	٢.٠٠ fg	١.٩٩ gh	٢.٤١ e	٤.٣١ d	٤.٤٦ bc	٤.٥٠ ab	٤.٤٣ c	٤.٥٠ ab	٤.٥٢ a	٠.٠٥٣
١٨ August	٢.١١ l	٢.١٤ kl	٢.٢٢ J	٢.٧٣ f	٢.٧٩ e	٢.٨٣ de	٢.٤٥ i	٢.٥١ h	٢.٥٦ gh	٤.٨٨ c	٤.٩٢ abc	٤.٩٤ ab	٤.٨٩ bc	٤.٩٢ abc	٤.٩٦ a	٠.٠٥٠
٣٠ August	٢.١٣ m	٢.١٦ lm	٢.١٩ Kl	٢.٧٧ g	٢.٨١ fg	٢.٨٣ ef	٢.٤٩ j	٢.٥٣ ij	٢.٦٠ h	٥.٢٩ d	٥.٣٦ c	٥.٤٢ ab	٥.٣٨ bc	٥.٤٢ ab	٥.٤٧ a	٠.٠٦١
Mean	١.١٨	١.٢٣	١.٢٨	١.٦٨	١.٧٦	١.٨٧	١.٦٩	١.٧٦	١.٩٣	٣.٦٦	٣.٧٦	٣.٨٢	٣.٧٤	٣.٧٩	٣.٨٣	

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)
*L.S.R. Least significant range (Duncan range at ٥٪).

On the other hand results showed that acetyl salicylic acid resulted in the lowest infection percentages (١.١٨, ١.٢٣ and ١.٢٨٪) when carried on sugar powder, candy and sugar syrup, respectively, followed by Thymol and Terramycine mixed with sugar powder (١.٦٨ and ١.٦٩٪, respectively). However using potassium sorbate with sugar powder, candy and sugar syrup resulted in ٣.٦٦, ٣.٧٦ and ٣.٨٢٪ infection, respectively.

In summer ٢٠١١, results of Table (٩) and Figure (٧) showed that the efficacy of acetyl salicylic acid surpassed the efficacy of Terramycine which in turn surpassed the efficacy of Thymol, while the efficacy of potassium sorbate came in last position.

Time appearance and control of chalkbrood disease

Table ٩: Mean percentages of chalkbrood infection monitored during summer of ٢٠١١ in honeybee colonies previously treated in winter ٢٠١٠/٢٠١١ with the four tested chemicals at Minia region.

Treatments / Date	Acetyl salicylic acid			Thymol			Terramycine			Potassium sorbate			Control			*L.S.R at ٥٪
	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	Sugar Powder	Candy	Sugar syrup	
١ July	١.٠٨ o	١.١٠ no	١.١٦ m	١.٣١ l	١.٣٣ kl	١.٤٥ j	١.٦٦ i	١.٦٩ hi	١.٧٢ gh	٣.٠٢ f	٣.٠٥ ef	٣.١٣ bcd	٣.٠٩ de	٣.١١ cd	٣.٢١ a	٠.٠٥٠
١٣ July	٠.٢٥ n	٠.٣٣ m	٠.٣٩ l	٠.٨٦ k	٠.٨٩ jk	٠.٩٣ ij	١.١٣ h	١.١٨ gh	١.٢٢ fg	١.٩٦ e	٢.٠٢ cd	٢.٠٤ bcd	٢.٠٠ de	٢.٠٨ ab	٢.١٢ a	٠.٠٤٨
٢٥ July	٠.١٨ mn	٠.١٦ n	٠.٢١ lmn	٠.٢٢ klm	٠.٢٩ j	٠.٣٦ i	٠.٦٥ gh	٠.٦٣ h	٠.٧٨ f	١.٥٥ e	١.٦٠ cde	١.٧٩ a	١.٥٨ de	١.٦٢ bcd	١.٨٣ a	٠.٠٥٣
٦ August	٠.٨٦ o	٠.٩٣ n	١.٠٣ m	١.٢٣ l	١.٣٠ k	١.٣٨ j	١.٥٥ i	١.٦٢ h	١.٧٣ g	٣.٧٩ f	٤.٠١ d	٤.١٩ b	٣.٨٧ e	٤.٠٥ c	٤.٢٢ a	٠.٠٠٢
١٨ August	١.٩٣ j	٢.٠٣ ij	٢.١٥ hij	٢.٤٣ ghi	٢.٥٢ fgh	٢.٦١ efg	٢.٦٦ d-g	٢.٧٠ c-g	٢.٧٩ b-g	٤.٠٠ a	٤.١١ a	٤.٣٢ a	٤.٠٣ a	٤.١٩ a	٤.٣٥ a	٠.٤٤٣
٣٠ August	٢.٠١ o	٢.٠٩ n	٢.١٨ m	٢.٥٣ l	٢.٦٠ k	٢.٧٢ j	٢.٨٠ i	٢.٨٦ h	٢.٨٨ gh	٤.٧١ f	٤.٨٣ d	٥.٠٢ b	٤.٧٣ ef	٤.٨٧ cd	٥.٠٨ a	٠.٠٥٢
Mean	١.٠٥	١.١١	١.١٩	١.٤٣	١.٤٩	١.٥٨	١.٧٤	١.٧٨	١.٨٥	٣.١٧	٣.٢٧	٣.٤٢	٣.٢٢	٣.٣٢	٣.٤٧	

For each row, means followed by the same letters are not significantly different at ٥٪ level of probability (Duncan multiple range test)
*L.S.R. Least significant range (Duncan range at ٥٪).

Data in that season showed that the highest mean infection percentages recorded for using potassium sorbate treatment (٣.٤٢, ٣.٢٧ and ٣.١٧٪), followed by Terramycine (١.٨٥, ١.٧٨ and ١.٧٤٪), followed by Thymol (١.٥٨, ١.٤٩ and ١.٤٣٪), while using acetyl salicylic acid resulted in the lowest mean infection percentages (١.١٩, ١.١١ and ١.٠٥٪) when mixed with sugar powder, candy and sugar syrup, respectively.

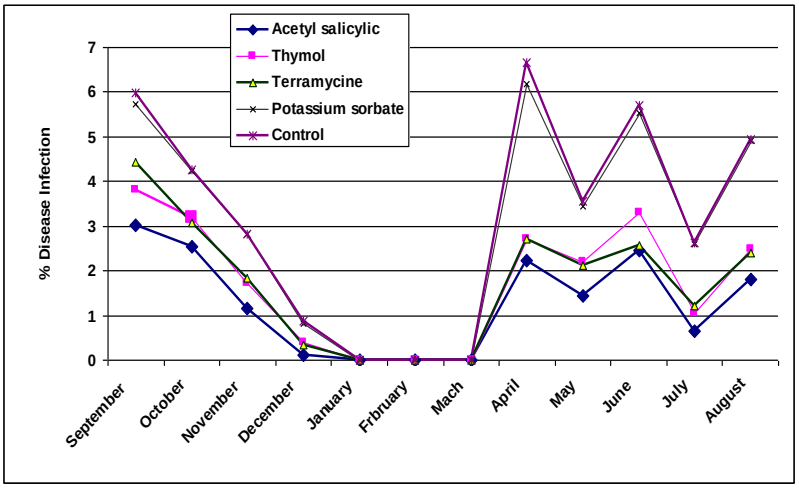

Fig ٦ : Mean percentages of chalkbrood infection monitored during the four seasons ٢٠٠٩/٢٠١٠ in colonies treated during autumn and winter of ٢٠٠٩/٢٠١٠ Terramycine and other three chemicals at Minia region.

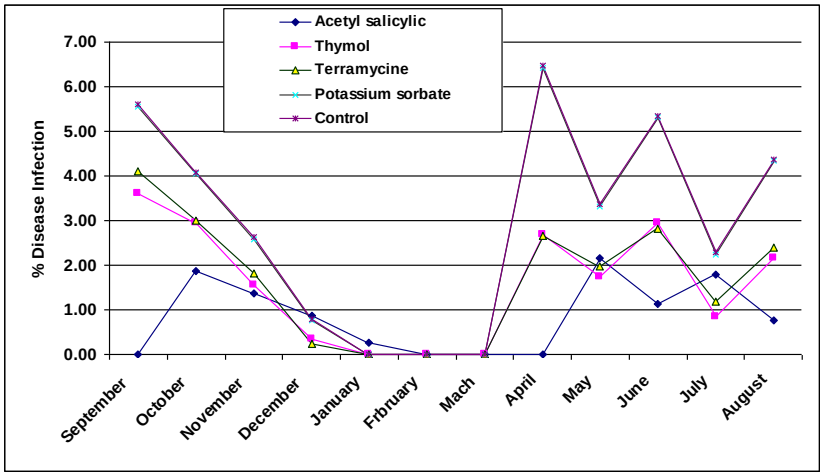

Fig ٧ : Mean percentages of chalkbrood infection monitored during the four seasons ٢٠١٠/٢٠١١ in colonies treated during autumn and winter of ٢٠١٠/٢٠١١ Terramycine and other three chemicals at Minia region.

Statistical analysis of the obtained data showed that there were significant differences among efficacy of different treatments.

These results are in agreement with those results obtained by Calderone *et al.*, (١٩٩٤) who found that Thymol inhibited fungus growth. Spivak and Reuter, (٢٠٠١) and Davis and Ward, (٢٠٠٣) found that using oxytetracycline gave excellent results and reduce the infection level of fungus disease to ٣٠٪ at summer (by ٣٩٪reduction). Flores *et al.*, (٢٠٠٤) reported that oxytetracycline reduced infection percentage up to ٤٢٪ of chalkbrood in the honeybee (*Apis mellifera* L.).

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دراسات على توقيت ظهور ومكافحة مرض ألحضنه الطباشيري في منطقة المنيا

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تم إجراء هذه الدراسة في منطقه المنيا وذلك في الفترة الممتدة بين عامي ٢٠٠٩ إلى ٢٠١١م حيث استخدمت طوائف نحل عسل متساوية قي القوه من سلاله النحل الكرنيولي الهجين الأول ويغطي النحل ٨ أقراص شمعيه في المتوسط ، وتم استخدام طوائف تحتوي على ملكات أخوات ملقحه ، وكذلك مخزون غذائي متساوي.

أوضحت النتائج أن أعلى نسبة مئوية للإصابة بمرض ألحضنه الطباشيري (٧.٢٥%) تم تسجيلها في ٢٠ ابريل (متوسط درجة حرارة ٣٢.٤ درجة مئوية ، ٦٩.٦% رطوبة نسبية). بينما كانت اقل نسبة إصابة (تتراوح بين صفر – ٠.٦٦%) خلال الفترة الممتدة بين ديسمبر ٢٠٠٩ حتى نهاية مارس ٢٠١٠م (متوسط درجه حرارة ١٨.٣٠ – ٢٥.٨ درجة مئوية ورطوبة نسبية تتراوح بين ٦٥.٥ – ٧٧.١%).

وتم استخدام ثلاث مركبات كيميائية (حامض الأستيل ساليسليك والثايمول وسوربات البوتاسيوم) والتيراميسين كمضاد حيوي في ثلاث صور تغذية مختلفة (سكر بودرة – كاندى – محلول سكرى).

وأوضحت النتائج أن إستخدام حاض الأستيل ساليسليك أعطى أعلى خفض فى % للإصابة بمرض الحضنة الطباشيرى (أعلى من ٥٢.٥٤ % فى الخريف ، ٨٠.٤١ % فى الشتاء) ولكن التيراميسين جاء فى المرتبة الثانية حيث وصلت نسبة الخفض الى ٣٣.٢٦% فى الخريف ، ٨٠.٠٧% فى الشتاء . بينما شغل الثيمول المستوى الثالث والذى أدى الى خفض للإصابة بهذا المرض حوالي ٣٦.٩١% فى الخريف ، ٧٥.٠٩% فى الشتاء. فى حين استخدام سوربات البوتاسيوم لم يعطى نتائج مرضية ضد هذا المرض حيث كانت حوالي ٣.٣% فى الخريف ، ٣٧.٧٢% فى الشتاء.