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STUDY ON THE ANTIMYCOPLASMAS ACTIVITY AND BROILER PERFORMANCE OF HONEY BEE

(With 4 Tables)

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دراسة عن تأثير عسل النحل على معزولات ميكروب الميكوبلازما

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أوضحت الدراسة أن عسل النحل فى تركيز ١٢,٥% أو أكثر يقتل ميكروب الميكوبلازما جاليسيبتكم بالكامل بينما عند تركيز ٦,٢٥% له بعض التأثير على نفس الميكروب والتركيزات التى أقل من ٦,٢٥% ليس لها تأثير على ميكروب الميكوبلازما . وبدراسة مقارنة بين الأدوية المضادة للميكوبلازما والعسل عن طريق الأقراص المشبعه بالعسل ١٢,٥% والأقراص المعايره من بعض المضادات الحيوية وجد أن للعسل تأثير وسط على ميكروب الميكوبلازما مشابهة تقريبا لتأثير عقار الأرثروميسين أو الأسبيراميسين على نفس الميكروب ولكنه أقل فى التأثير من عقار الأنروفلوكساسين .أوضحت الدراسة أن كتاكيت التسمين المعدية صناعياً بميكروب الميكوبلازما جاليسبتكم في المجموعة المعالجة في ماء الشرب بعسل النحل ١٢,٥% والمجموعة المعالجة بالبايتريل (١٠ ملجم / ك) والمجموعة المعالجة بعسل النحل والبايتريل إيجابية لإختبار التلازن للميكوبلازما عند عمر ٣٥ يوم بنسب ١٤% و ١٢% و ٦% في تلك المجاميع بالتتابع هذا ولم يتمكن من إعادة عزل الميكروب من الدجاج النافق والإيجابي بإختبار التلزن لميكوبلازما الدجاج. هنا وقد دلت الدراسة علي أن استخدام عسل النحل مع عقار البيتريل (إنروفلوكساسين) يزيد من كفاءة معدلات التحويل الغذائى فى كتاكيت التسمين ويقلل من نسب النفوق.

SUMMARY

The antimycoplasmas activity of honey bee was investigated. A complete growth inhibition of *Mycoplasma galisepticum (MG)* was a achieved at concentration of 50, 25 and 12.5% honey bee, a partial inhibition was at 6.25% honey bee while all concentration less than 6.25% honey bee achieved no effects on *MG*. On biogramme study of some antimycoplasmas drugs and honey bee, the results showed

moderate effects of honey bee more or less similar to the effects of erythromycine and spiramycin on MG. In *vivo* study, honey bee 12.5% plus Baytril (10 mg/kg) was found to be more effective when medicated to broiler chicks artificially inoculated with MG. 6% serological reactors at age of 35 days and feed conversion rate 1.9 were achieved by honey bee plus Baytril medication programme followed by Baytril (12% serological reactors at age of 35 day and feed conversion rate 2.05%) and honey bee 12.5% alone (14% serological reactors at age of 35 day and feed conversion rate 2.1) when compared to control infected non medicated (78% serological reactors at age of 35 day and feed conversion rate 2.82). MG organism could not be reisolated from dead and serologically reactor chicks.

Key words: *Mycoplasma, broiler, Honey bee.*

INTRODUCTION

Honey bee is widely used in folk-medicine throughout the world (Jarvis, 1961). Honey is considered not only as a sweetener but also as a healthy and wholesome food with curative properties. It has a direct nutrient effect on regenerating tissues as it contain a wide range of amino acids, vitamins and trace elements in addition to large quantities of readily assimilable sugars (Molan, 1998). It has therapeutic properties and has been used to cure diseases of the respiratory system, intestinal and cardiovascular diseases (Yaniv and Rudich, 1996). It is used with good effect as dressings for wound infections and ulcers (Cooper and Molan, 1999 and Efem *et al.*, 1992). Honey acts as an antimicrobial agent against many bacteria as Staphylococcus aureus (Mishref *et al.*, 1989; Allen *et al.*, 1991 and Molan, 1992) ; H.pylori (Ali *et al.*, 1991 and Osato *et al.*, 1999), Pseudomonas spp. (Cooper and Molan, 1999) and the pathogenic black pigmented bacteroides melaninogenicus (Elbagoury and Rasmy, 1993). Omayma (1998) showed that S.typhimurium, E.coli, Proteus mirabilia, Yersinia enterocolitica and Pseudomonas aeruginosa were inhibited by 25% honey bee. Moreover, the antimicrobial action of honey bee appeared to be effective against antibiotic resistant strains of bacteria (Molan, 1998).

Mycoplasma infection and its complication in poultry industry still a serious problem, specially in hot climates (El-Batrawi *et al.*, 1990).

Although the periodical use of antimycoplasmas drugs can reduce the losses caused by mycoplasma infection. The problem was

never solved was never solve, as the organism can develop resistance against the drug after some time of its use (El-Batrawi *et al.*, 1990). The antibacterial activity of honey bee against some bacteria encourage us to study the activity of honey bee against MG in vitro and in vivo, as well as its effect on the performance of broiler chicks.

MATERIAL and METHODS

Honey bee:

Samples of crude honey bee were obtained from local market. It originated from hives located in Aga district Barsim growing area of Dakahlia province, Egypt.

Preparation of honey bee solutions:

The amounts of honey bee necessary to achieve the required concentration were aseptically weighted in sterile bottles and heart infusion broth was placed in each bottle to make up the total volumes of required concentration [3.12% to 50% (w/v)].

Preparation of honey bee discs:

Sterilized Whattman filter paper discs (5.6mm diameter) were soaked into appropriate concentration of honey bee (12.5%) and left to dry at room temperature, then stored in labeled dated and tight container in the refrigerator at 4°C till used (each disc contain 1.25 mg honey bee).

Baytril®:

It is a trade name of enrofloxacin 10% solution kindly supplied from Bayer, Germany.

Antibiotic sensitivity discs:

Enrofloxacin, spiramycin and erythromycin discs were supplied from Oxoid, meanwhile lincospectine discs were obtained from Neo-Sensitab Rosco Denemark.

Microorganisms:

Pure culture of MG was obtained from Mycoplasma Department at Animal Health Research Institute, Dokki, Giza.

Mycoplasma gallisepticum stained antigen:

It was supplied from Intervet Comp., Holland.

Inoculum preparation:

Stoke agar block of MG was subcultured for three times onto fresh Heart infusion agar plates (Biolife, Italy). Then agar blocks were transferred directly into broth isolation media, Heart infusion broth (Yoder, 1980). After 2 – 3 day incubation at 37°C, the actively young broth culture was used as working inoculum.

Determination of minimum inhibitory concentration (MIC) of honey bee on Mycoplasma gallisepticum:

Broth dilution susceptibility test was performed using different honey bee concentrations (3.15 to 50% w/v) and sterile heart infusion in sterile screw capped bottles. 0.2 ml of MG suspension was added to each bottle and incubated at 37°C. After 72 hours incubation, 0.1 ml of incubated broth from original and all dilutions were plated in duplicate onto Heart infusion agar plates and incubated in moist candle Jar at 37°C for 24–72 hours. The incubated plates were examined using dissecting microscope to evaluate the growth of mycoplasma colonies. The plates which showed no MG growing colonies was considered to be MIC.

Sensitivity of Mycoplasma gallisepticum to honey bee and some antibiotics:

A loopfull of young broth culture of MG was directly cultivated onto Brain heart infusion agar plates (Biolife, Italy) using the running drop technique. The plates were left to dry at room temperature. The freshly prepared honey bee disc and tested antibiotic discs were gently pressed in the middle of the inoculated streaks. The plates were incubated in moist candle jar at 37°C. After 3 days, incubation, the results were interpreted by measuring the zone of inhibition in mm from the edge of the disc to the microbial growth using the dissecting microscope (Bebear and Roberton 1996).

***In vivo* experiments:**

Two hundred and fifty, 7 days old apparently healthy broiler Hubberd chicks serologically negative for *Mycoplasma gallisepticum* were supplied from El-Mansoura Poultry Company, Dakahlia Province. The chicks were divided into 5 groups consisting of 50 birds each. The chicks were housed in separated room on deep Litter system and fed ad libum on a commercial balanced feed. Birds of group 1, 2, 3 and 4 were infected by intranasal instillation with 0.2 ml of fresh 24 hours mycoplasma-broth containing 0.5×10^8 viable organisms per chick (Suzuki *et al.*, 1970). Meanwhile birds of group 5 were kept as control (non infected). Chicks in group 1 was supplemented with honey bee solution 12.5% for 3 successive days, group 2 supplemented with Baytril in a dose of 10 mg/kg b.w., also for 3 successive days, while group 3 were supplemented with a honey bee 12.5% solution + Baytril 10 mg/kg B.W. for 3 successive days directly after chicks inoculated with MG. Same doses were repeated for three days at age of 21, 22, 23 days and 35, 36, 37 days, group 4 was inoculated but not treated by any drug and group 5 was kept as non inoculated non medicated group. The

vaccination programme for all birds was applied and whenever necessary antibacterials known to have no effect against mycoplasma were chosen according to sensitivity tests and used for all groups at the same time.

Sampling:

Random blood samples were collected from the wing vein of 25 (10%) of chicks before inoculation and from all birds at weekly intervals post inoculation for testing MG using rapid serum plate agglutination test according to Yoder (1980).

Samples from trachea, lung, heart blood and air sacs were taken from dead birds and serologically positive chicks for reisolation of mycoplasma.

The mortality rate, the average body weight and feed conversion rate were calculated at the end of experiment for all groups.

RESULTS and DISCUSSION

Vitro studies indicated that MG was completely inhibited by honey bee at concentration of 12.5% while 6.25% concentration had a partial effect on MG and all concentration of honey bee less than 6.25% had no effect on MG (Table 1). Many investigators recorded that honey bee had different degrees of inhibition on various species of bacteria. Willix *et al.*, (1992) found that the growth of *Staphylococcus aureus* was completely inhibited by honey bee concentration of 1.8% (v/v) during incubation for 8 hours. Mishref *et al.*, (1989) mentioned that complete bactericidal action against *S.aureus* would be acheved by the use of 1.3 to 5% honey bee concentrations. Cooper and Molan (1999) reported that MIC of the pasture honey bee on pseudomonas spp. was 7.1% (v/v) and of manuka honey bee on the same organisms was 6.9% (v/v) in average, moreover, Ali *et al.*, (1991) proved that growth of all Hilicobacter pylori isolated were inhibited by 20% honey bee concentration.

Table 1: Minimum inhibitory concentration (MIC) of the honey bee on *Mycoplasma gallisepticum*

Strain	Honey bee concentration					*Mycoplasma suspension*
	50	25	12.5	6.25	3.12	
Mycoplasma gallisepticum	-	-	-	++	+++	+++

- No growth
++ Moderate growth
+++ Heavy growth

In general the antimicrobial activity of honey bee is partially due to its osmolarity, acidity or due to hydrogen peroxide (Molan, 1992). The presence of antimicrobial substances, inhibines in honey bee which include not only hydrogen peroxide but also flavonoids and phenolic acid (caffeic acid & ferulic acid) (Wahdan, 1998) may be the other explanation of its effect.

The results of biogramme studies of honey bee and some antimycoplasma drug showed that honey bee in concentration of 12.5% has similar effect as erythromycin 25 µg / disc and spiromycin 100 µg / disc. While it has lesser effect than enrofloxacin 10 µg / disc or Lincospectin 15 µg/ disc on MG culture (Table 2).

Table 2: Biogram of some antimycoplasmas drugs and honey bee against *Mycoplasma gallisepticum*

	Disk concentration	*Sensitive*	*Intermediate*	*Resistant*
Honey bee	125 µg		++	
Enrofloxacin	10 µg	+++		
Lincospectine	15 µg	+++		
Erythromycin	25µg		++	
Spiramycin	100 µg		++	

Effect of honey bee and or Baytril on reducing MG infection level of experimentally inoculated chicks are shown in Table 3. 10% and 14% of chicks were positive in SPA test at age 21 and 35 days respectively when treated by honey bee. Meanwhile 8% and 12% of chicks were positive reactors at age 21 & 35 days respectively when treated by Baytril. Moreover, the number of positive reactors were greatly reduced at age 21 days (4%) and 35 days (6%) when both honey bee plus Baytril were used in treatment. These results indicate the synergistic effects of honey bee and Baytril on MG artificially inoculated broiler chicks.

Table 3: Results of serum plate agglutination test on artificially inoculated chicks

Groups	*Number of chicks*	*SPA test*	
		Day 21	Day 35
Honey bee	50	10%	14%
Baytril	50	8%	12%
Honey bee + Baytril	50	4%	6%
Infected non treated	50	62%	78%
Non infected non treated	50	0	0

SPA – Serum plate agglutination test

The synergistic effects of honey bee with Baytril may be attributed to the stimulation of immune system of chicks by honey bee (Shamala *et al.*, 2000) which may potentiate the antimicrobial effects of both honey bee and Baytril.

In a trial for reisolation of MG from trachea, lung, heart blood and air sacs of all serologically reactors and dead birds, we failed to isolate this organism. This finding agreed to those reported by Omuro *et al.*, (1970) and Singab, (1987) who reported that MG organism could not be recovered from body tissues of inoculated birds for 9 weeks and 6 weeks after inoculation respectively.

The study of effects of honey bee and/or Baytril on the performance of broiler chicks artificially infected with MG showed that mortality rate was 8, 4, 6, 21 and 12% in the tested groups treated with honey bee, honey bee + Baytril, Baytril, infected non treated and non infected non treated chicks respectively. The results showed also that feed conversion ratio (F.C.R.) was 2.1, 1.9, 2.05, 2.80 and 2.22 for the same groups respectively (Table 4).

Table 4: Effect of honey bee and/or Baytril on performance of broiler chicks at age 42 day

Groups	Mortality %	Feed consumption (kg)	Body weight (kg)	Feed conversion rate (BCR)
Honey bee	8	4.200	2.00	2.1
Honey bee + Baytril	4	4.00	2.100	1.9
Baytril	6	4.100	2.00	2.05
Infected non treated	21	3.100	1.100	2.82
Non infected non treated	12	4.00	1.800	2.22

The lowest mortality rate (4%) and highest F.C.R. (1.9%) in the group treated with honey bee and Baytril may be due to the alteration of microbial profile of the intestine with special reference to a significant increase in lactic acid bacteria which in turn would influence the physiology and health of the host animal, in addition to this, honey bee has antibacterial substances which also import additional benefits (Shamala *et al.*, 2000).

In conclusion, according to the results of our study which revealed the synergistic effect of honey bee with Baytril, honey bee can be use as adjuvant enrofloxacin as well as with other antimicrobial drugs for improving its potency and for stimulation of immune system of chicks.

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