



الخطة الوطنية للعلوم والتقنية  
The National Plan for Sciences & Technology

# Role of External Immune Stimulation and Its Impact on the Honey Bee *Apis mellifera jementica* in Saudi Arabia

Hend M. Alharbi , Ahmad Al Ghamdi , Ashraf M. Ahmed & presented by : *Tahany H. Ayaad*



**1- Mrs**

**Hend M. Alharbi**

**Department of Zoology, College of Science, King Saud University,**

**2-Professor**

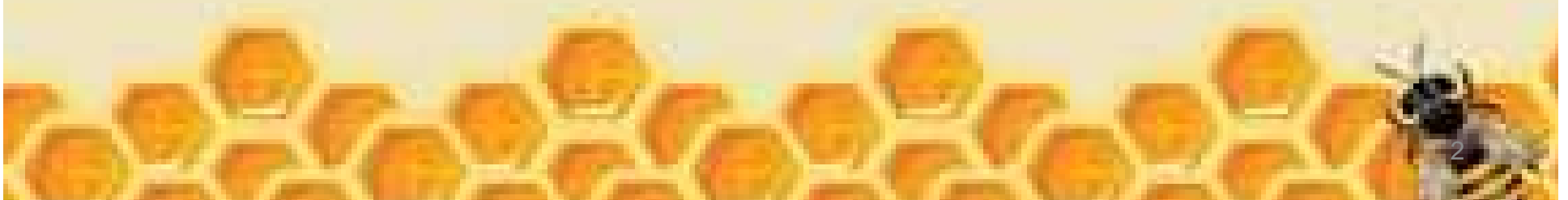
**Ahmad Al Khazim Al Ghamdi**

**Chair of Engineer Abdullah Ahmad Bagshan for Bee Research, Bee  
Research Unit, Plant Protection Department, Faculty of Food  
Sciences and Agriculture, King Saud University.**

**3- Professor**

**Ashraf M. Ahmed**

**Department of Zoology, College of Science, King Saud University**



➤ **Honeybees are one of the well-known economic beneficial insects known to man from time immemorial .**

➤ **In Saudi Arabia Beekeeping contributes to the traditional economic development of the country by providing sustainable additional income through self employment.**



Honey

Pollen

Beewax

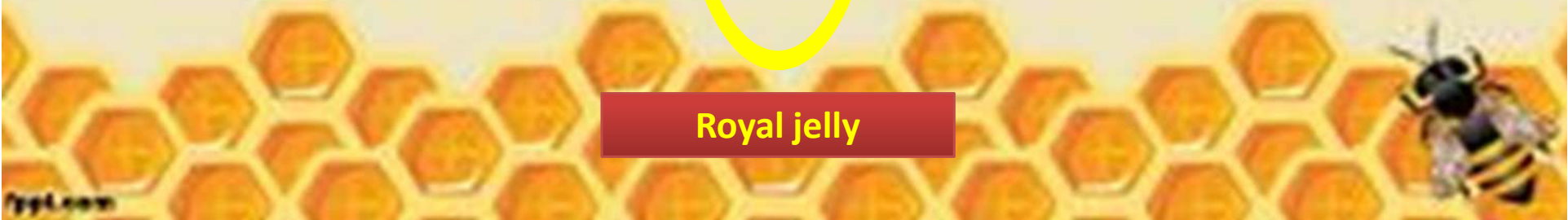
Propolis

Royal jelly

Bee venom

pollination

Antimicrobial peptides



# Bees Hive Products



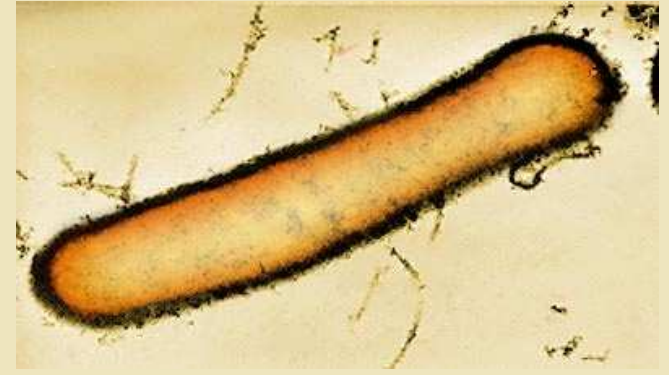
Fig. 1. The distribution of *Apis mellifera jemenitica* in Africa and Asia (all areas within bold line) based on published reports (Ruttner, 1988; Hepburn & Radloff, 1998).



➤ *Apis mellifera jementica*  
(*A.m.jementica*) in KSA shows  
high adaptation to the  
environmental conditions in  
the west, south and south west  
regions during the hot summer  
at temperature exceeds 45 °C.



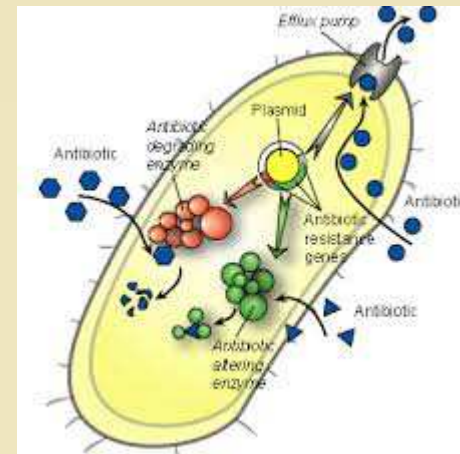
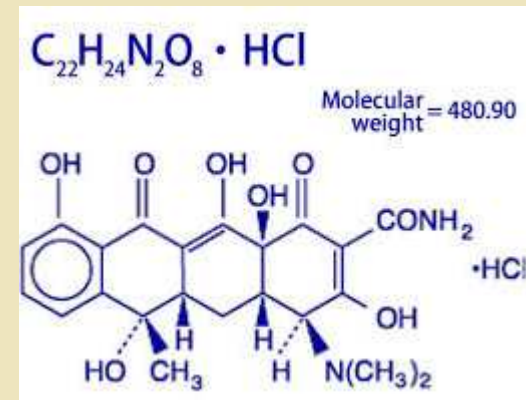
- Honey bee brood and adults are attacked by bacteria, viruses, protozoans, fungi and parasitic mites.
- The American Foulbrood (AFB) is an infectious worldwide apiculture threatening bacterial disease of larvae and pupae transmitted by the Gram negative *Panaebacillus larvae* (*P.larvae*).







- Resistance of *P. larvae* bacteria to the currently used conventional antibiotics [e.g. oxytetracycline, Terramycin and tetracycline] maximized the Apiculture threat by this disease that impose urgent demands for new classes of therapeutic antibiotics.

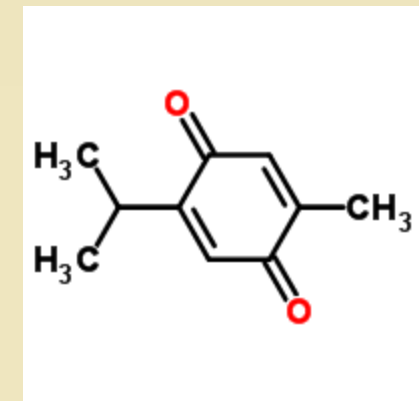
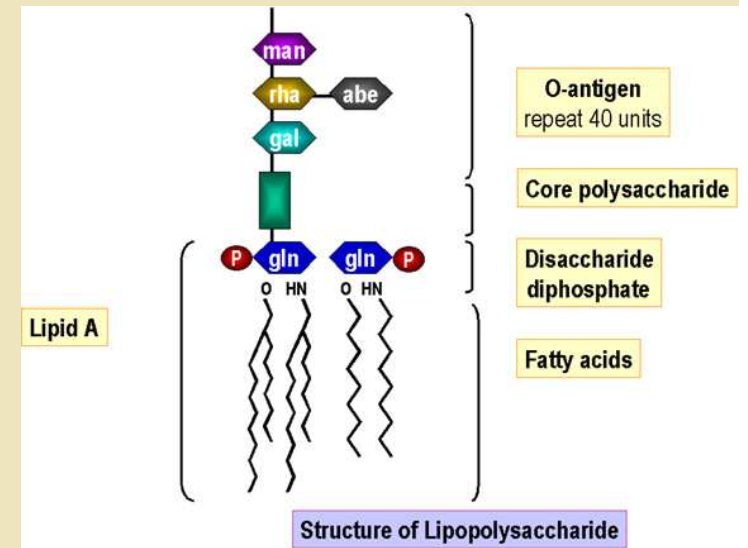


## Research Objectives

- **The current research is a ground step of a project entitled “Humoral immune responses of the native honeybee, *Apis mellifera jementica* towards developing natural antibiotics against infectious food-borne diseases“**
- **This project is funded by the National Plan for Science and Technology of King Saud University (project code number ASTP-10 ) .**



- Research aims herein focuses on induction of the humoral immune response of worker *A.m. jementica* in response to injection of lipopolysaccharides (LPS) or *Micrococcus luteus* (*M.luteus*) simulating the action of bacterial infection.
- Bees are fed on standardized dose of Thimoquinone TQ for 72 hrs, then injected with LPS or *M. luteus*



Thimoquinone Structure

2-Isopropyl-5-methyl-1,4-benzoquinone



- **AMPs elicited in worker hemolymph treated groups are evaluated in vitro by an agar well diffusion assay against *E. coli* as Gram-negative bacteria or a panel of Gram positive – bacteria.**

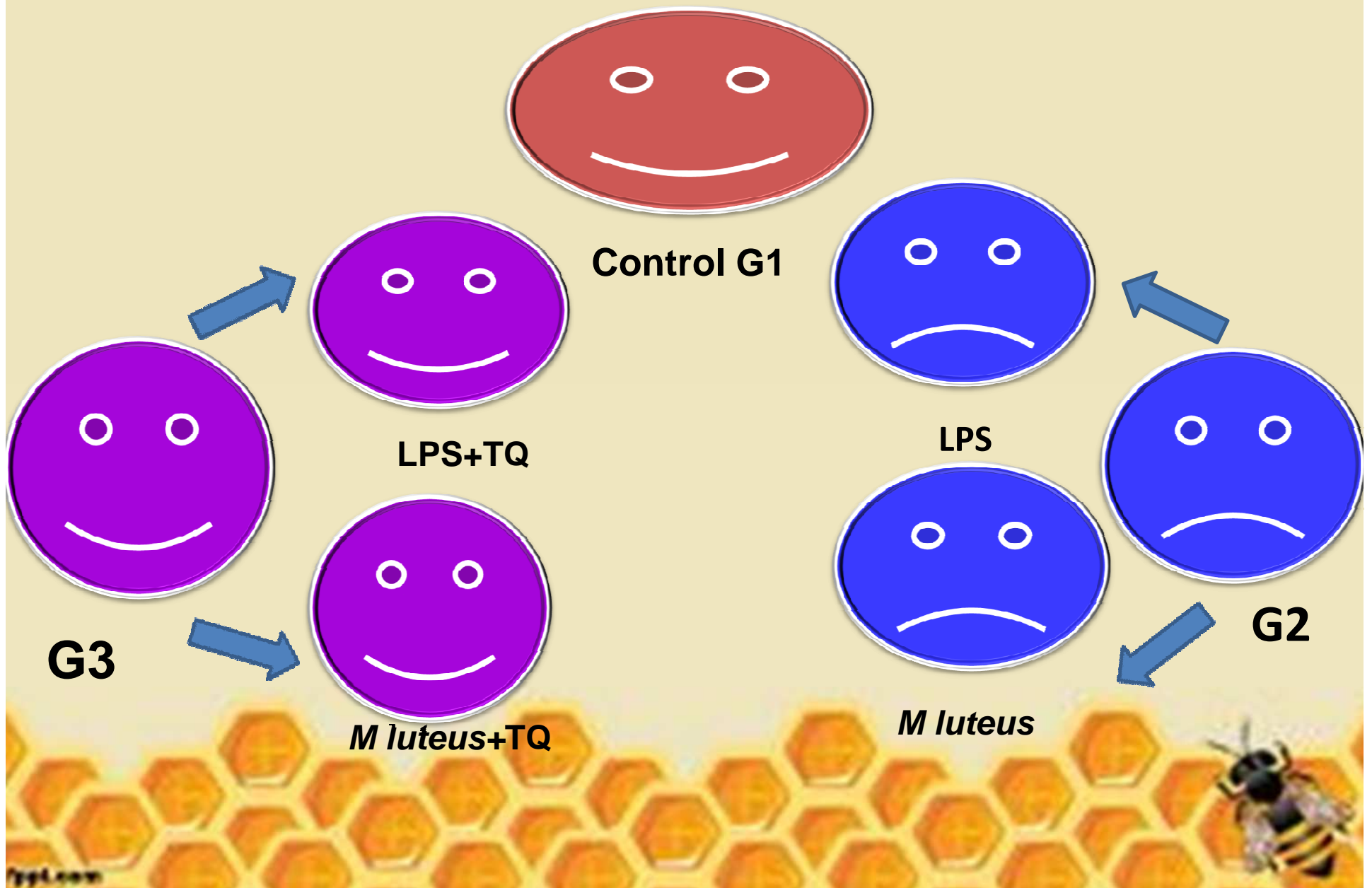




**Examples of apiaries with local hives established in open fields or in constructions**



# Experimental groups (Gs)



- **An aliquot (0.2 ml) of a fresh`overnight culture was spread onto agar plates (Ø59 cm) containing NB and LB medium, respectively.**
- **As soon as the bacterial layer had been adsorbed, 1.5 ml of undiluted hemolymph samples were applied as a droplet onto the plates with a pipette tip.**
- **After 24 h of incubation in an incubator at 37°C, the diameter of the clear zone of inhibition was measured and documented by photography.**





- **Zone-inhibition assay for the detection of antimicrobial activities in the hemolymph of infected bees were done.**
- **Aliquots of fresh overnight cultures of the Gram- negative or Gram-positive bacteria (0.2ml) were spread on agar plates.**
- **Hemolymph aliquots (50  $\mu$ l) directly applied onto the agar plate and incubated overnight at 37°C.**





Results

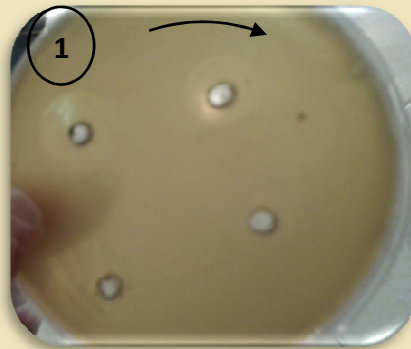


## Statistical Analysis

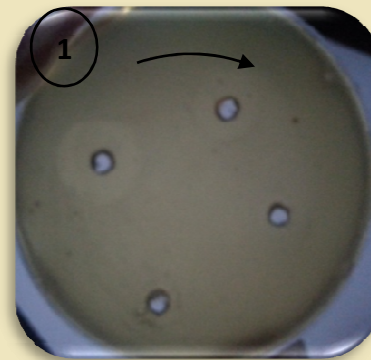
➤ Results were statistically analyzed using software (SPSS) version number 12 (One-Way Analysis of Variance ANOVA) and the comparison between the groups is significant with high P value  $\leq 0.025$  and insignificant if the value  $P \geq 0.025$ .

# Zone Inhibition Assay for detection of antimicrobial activity in hemolymph of LPS injected *A.m. jementica* towards *E.coli*

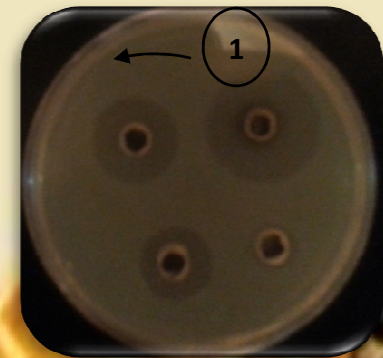
Control



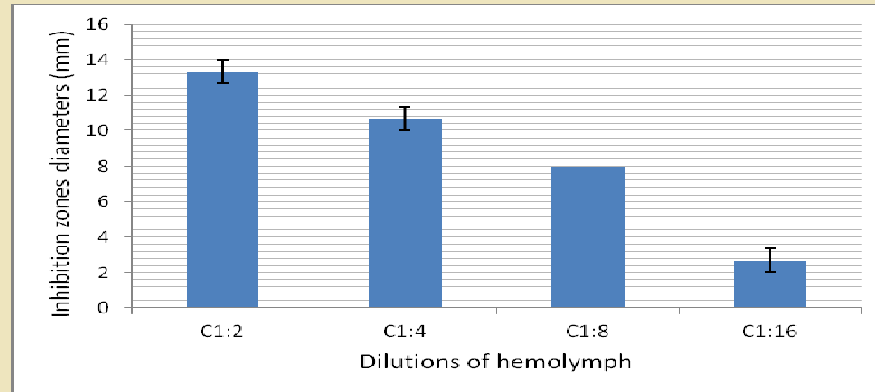
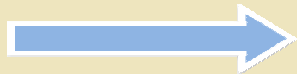
LPS injection



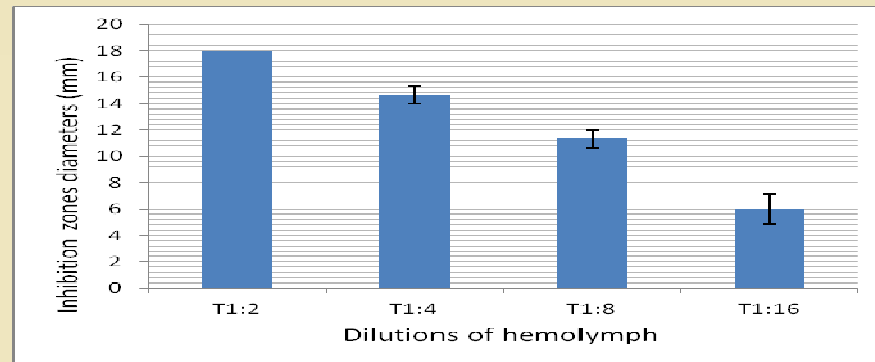
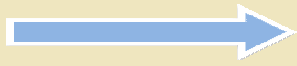
TQ+ LPS



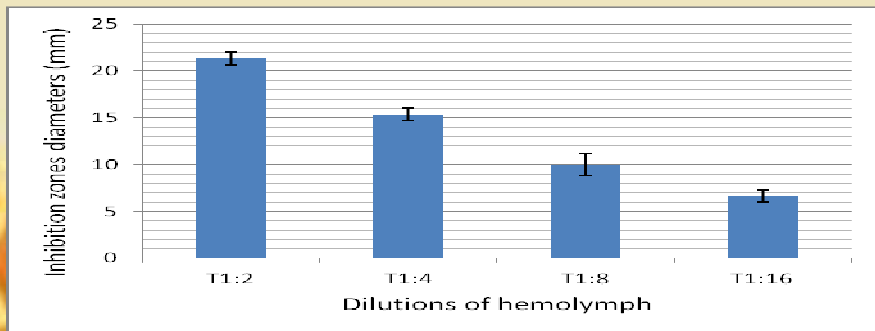
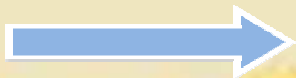
**control**



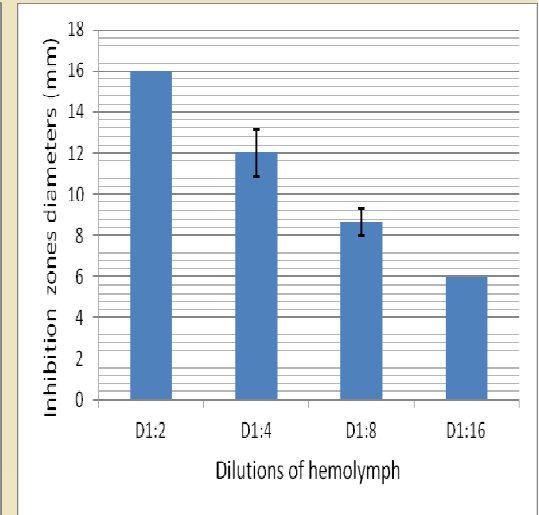
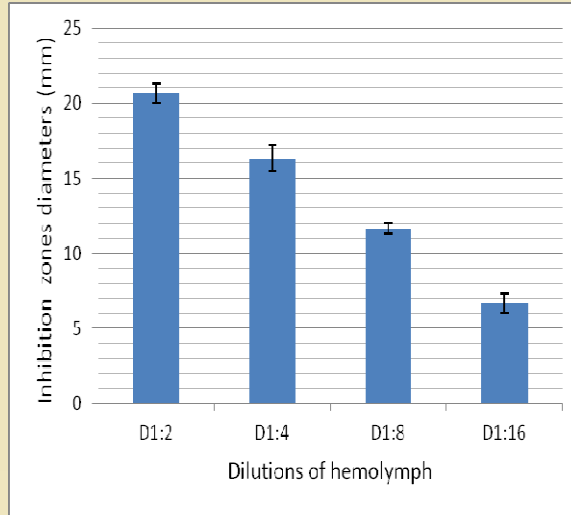
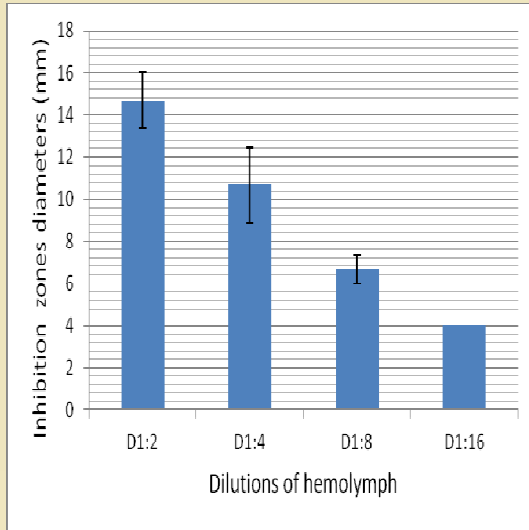
**LPS injected**



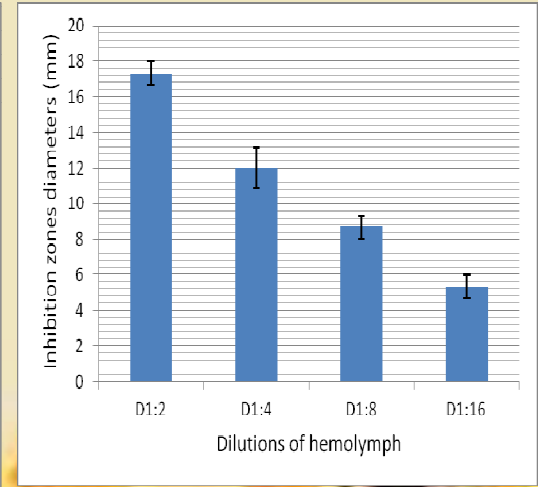
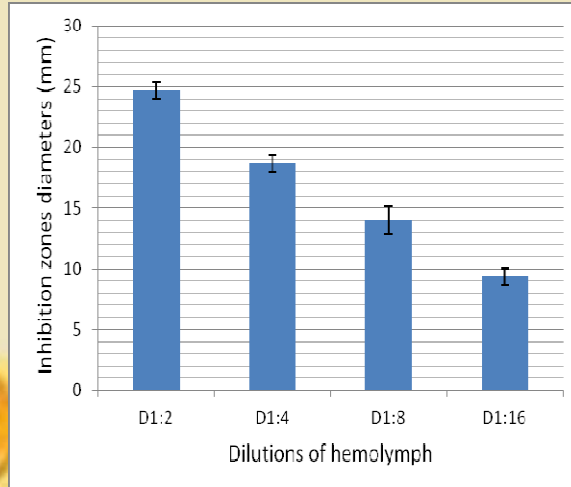
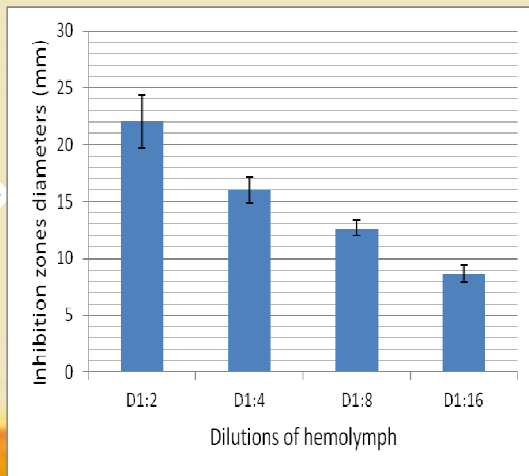
**TQ fed +  
injectionLPS**



LPS injection



TQ + LPS injection



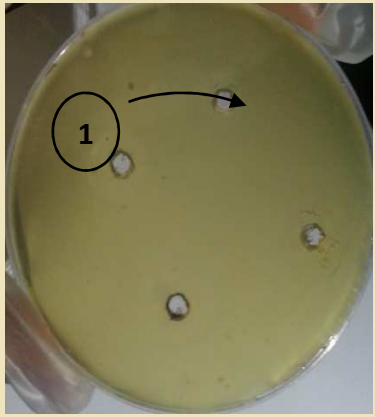
*B. subtilis*

*M. luteus*

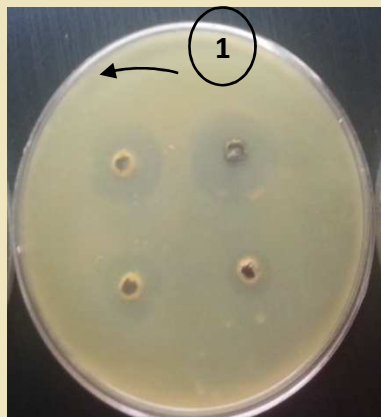
*St. aureus*

# Zone Inhibition Assay for detection of antimicrobial activity in hemolymph of *M.luteus* injected *A.m. jementica* towards *E. coli* bacteria

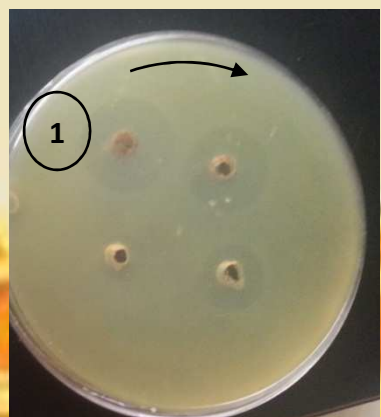
Control



*M.luteus* injection

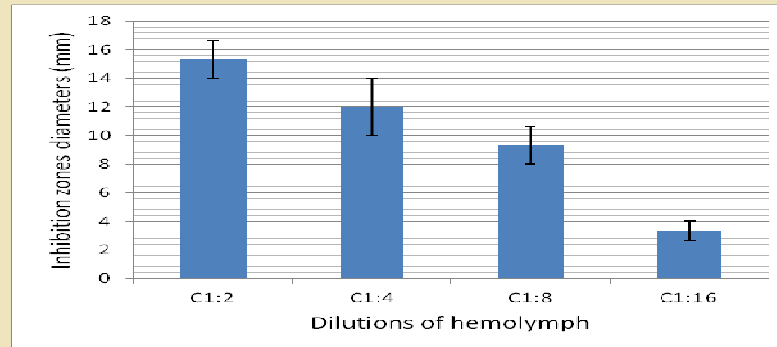


TQ+ *M.luteus*

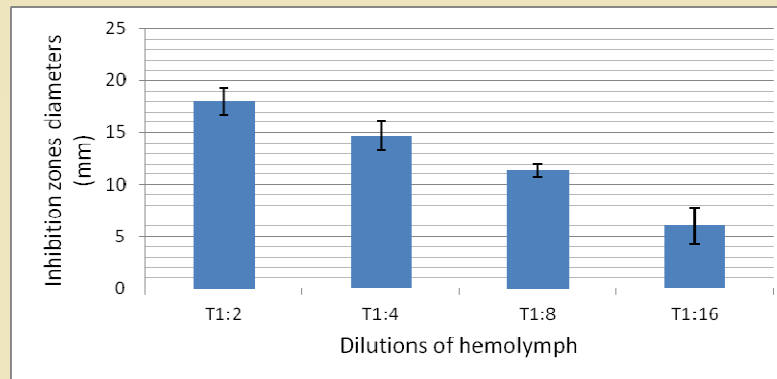


# Zone Inhibition Assay for detection of antimicrobial activity in hemolymph of *M.luteus* injected *A.m. jementica* towards *E. coli* bacteria

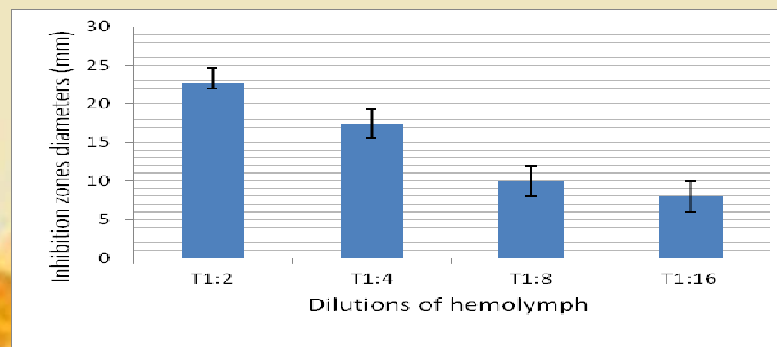
Control



*M.Luteus*



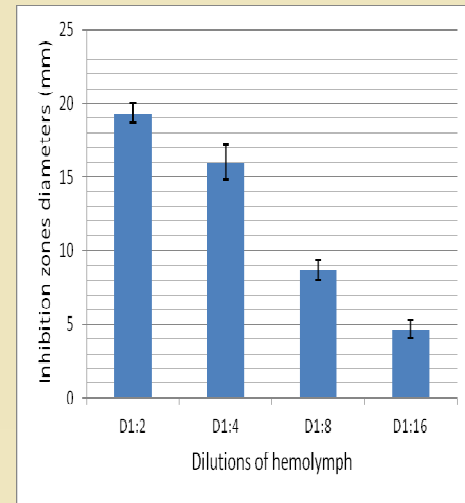
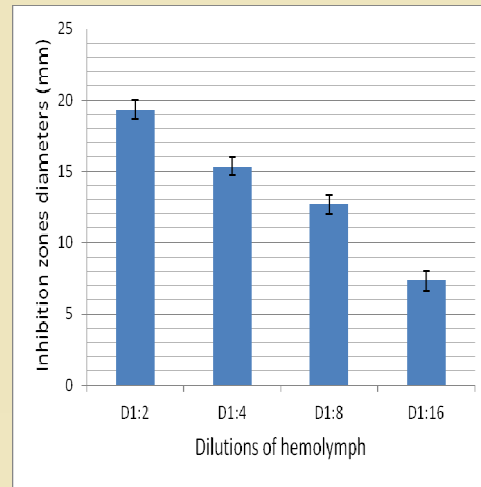
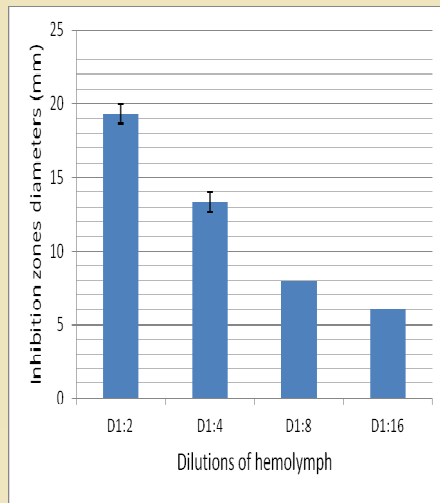
TQ+ *M.Luteus*



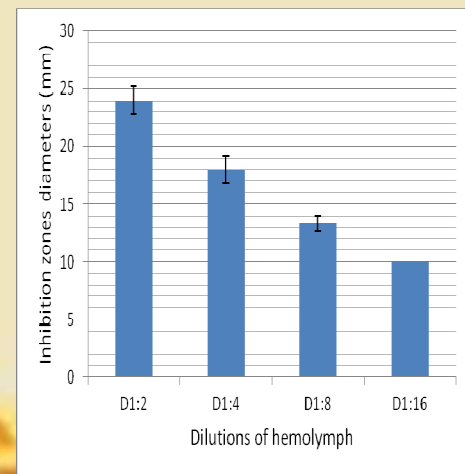
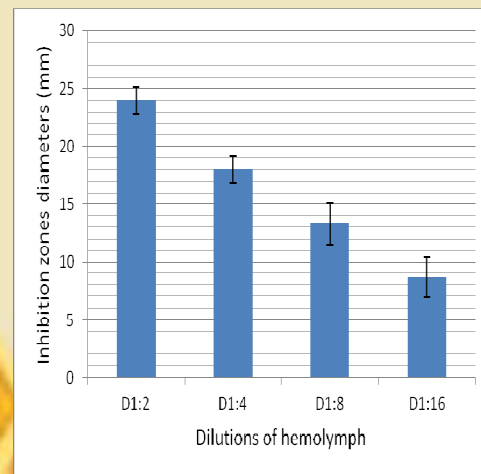
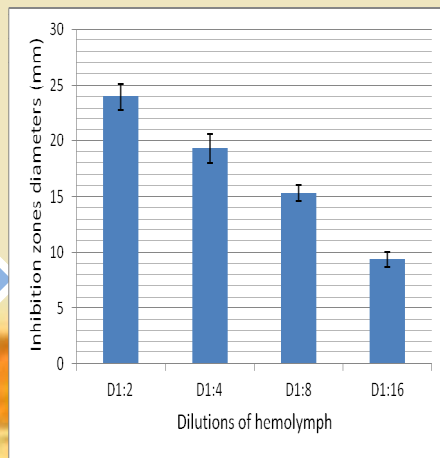


# Zone Inhibition Assay for detection of antimicrobial activity in hemolymph of *M.luteus* injected *A.m. jementica* towards different gram positive bacteria

*M.Luteus*



TQ+  
*M.luteus*



*B.subtilis*

*M.luteus*

*St. aureus*

- **The obtained data are in good agreement with Miyagi *et al.* ( 2000) in which they reported that treatment of bees with fungicides and antibiotics leads to the suppression of the bees' immune system, the emergence of resistant pathogens, and the contamination of bee products .**
- **Enhancement of the honey bees immune system by increasing the expression level of AMPs in bees themselves, is the solution to these problems (Bilikova *et al.*, 2001).**
- **Shen *et al.* (2010) have confirmed and chemically synthesized a proline rich peptide (PPO30) active towards both Gram negative and Gram positive bacteria**



# Conclusion

- **The current study could be used to build on approaches to enhance the immune system of this native honeybee to withstand the extreme environment of KSA, as well as developing protective therapeutic antibiotic(s) *via* the recombinant technology to control the most threatening bees food-borne diseases.**





## *Acknowledgment*

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10 )**



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Thank You

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