# Influence of *Peganum harmala* extract on the growth of some bacteria and *Fusarium acuminatum* producting T-2 toxin .

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## Abstract :

**The antimicrobial action of various concentrations of *Peganum harmala* extracts on growth of four isolates of pathogenic bacteria and toxigenic isolate of *Fusarium acuminatum* producing T-2 toxin were studied . The results showed that gram positive bacteria ( *Staphylococcus aureus* and *Bacillus subtilis* ) were more sensitive to different concentrations ( 125 – 500 mg /Disk) of harmal extracts than gram negative bacteria ( *Escherichia coli* and *pseudomonas fluorescens* ) . *S. aureus* was highly affected than the other bacteria . The results obtained during this study also showed that the harmala extracts were highly active against both of *Fusarium acuminatum* growth and $T-_2$ toxin at the concentration of harmala which varied from 400 to 600 ppm which showed decreased in mycelial growth from 1.90 to 1.33 ug / 50 ml and from 119.1 to 96.5 ug / ml for T-2 toxin production in liquid medium after 20 days of incubation at 25 C .**

## Introduction :

**Several medical plants , spices and herbs are widely used in food today for their flavors and aroma . Some of plants and spices have been found to posses antimicrobial activities because they contain different compounds such as alcoholes , alkloids and phenols ( Shelef *et al* ., 1980 ) . which have inhibitory effect against some food-borne bacteria and fungi . Recent articles demonstrate the inhibitory properties of garilc , onion in addition to rosemary and cinnamon on the growth of some pathogenic bacteria or fungi that produce mycotoxin (Al-Delamiy and Ali, 1970 ; Shelef *et al* ., 1980 ; Farag and Abo-raya , 1989 ; Mohmmd , 1995 and 2004 ) .**

**Some articles refered to the antimicrobial activity of some medical plants and spices against fungi producing mycotoxins and some food – borne bacteria such as *Salmonella* , *S. aureus* as well as growth and toxin production of *Clostridium botulinum* . Very few reports are available on the effect of harmala extract against some bacteria and fungi ( Musa and Mohmmad , 1992 ) . *Pegaum harmala* is one of the herbs belong to family Zygohyllaceae and widely used by Iraqi people . The object of the present study is planned to examine the inhibitory effects of harmalla extract on the growth of four isolates of pathogenic bacteria and toxiyenic isolate of *Fusarium acuminatum* producting T-2 toxin in liquid medium .**

## Materials and Methods

### Prepreation of harmala extracts

***Peganum harmala* was purchased from local market in Basrah – Iraq . various concentrations ( 125 , 250 , 500 mg / Disk ) . were prepread by weight of 50 g of harmala seeds in 100 ml distilled water . The extraction was done in boiling temperature for 1–1.5 hrs (Hussain,1981) and the marcerates were filtered using a buchner funel and No. 1 Whatman filter paper . Then centrefugation was done ( 3000 r/min) for 10 min . The extracts were immediately stored at a refrigerator until used also 200 , 400 and 600 ppm of harmala extracts were preperad with liquid medium .**

### Antibacterial action

**The test bacteria were from Biology Dept. Basrah university , and were each preincubated in 10 ml of Nutrient broth ( oxoid ) for 18 hrs at 37 C . Bacterial solutions prepared by nutrient broth were diluted 5 times with sterilized physiological saline , streaking methods were done on the agar plate , disk – diffusion assay was used to study the effect of antibacterial of harmala extracts on the bacterial growth (El-Kady *et al* ., 1993). disks ( from Whatman No. 3 filler paper ) containing**

**various concentration of harmala extracts as show in table 1 , and the plats were incubated at 37 C for 24 h and the inhibition zones of bacterial growth were measured by capillary vernia ( mm ) .**

**Antifungal action**

***Fusarium acuminatum*** **procured from Biology Department college of science , university of Basrah was used for $T-_2$ toxin production in semisythetic medium ( YES ) broth (20 g yeast extract , 150 g sucros and 1000 ml distilled water ) . Various concentrations of harmala extracts were added to medium the finall concentrations were ( 0, 200 , 400 and 600 ppm ) The medium was inoculated with 0.05 ml of the spore suspension , the cultures were incubated in the dark at 25 C and diplicate cultures of each concentration were analyzed after 20 days ( Garies *et al* ., 1984 ) .**

**Determination of mycelial dry weight**

**At the end of the incubation period , mycelium was separated by filtration and its dry mass was determined after drying at 105 C for 12 h ( Tiwari *et al* , 1986 ) .**

**Fusario toxin analysis :**

**T–2 toxin was purified , identified and quantitated from liquid medium as described by Garies *et al*,(1984); Omurtag and Yazicioglu, (2001) by using thin layer chromotography and HPLC . Standard toxin of T – 2 . procured from Dr. Mirocha , C. J . , Department of plant pathology , University of Munsota .**

**Results and Discussion**

**1. Antibacterial activity**

**The results in Table 1 shows the inhibition zone for gram positive and negative bacteria tested against various concentration of harmala extract when it was added to medium, The data indicated that the concentration ( % 125 mg / Disk ) of harmala had no or little effects against gram negative bacteria , (*E.coli* and *psendomonas flourescens*) The inhibition zone for *E.coli* and Ps.flourescens were 2.1 and 2.0 mm respectively , and increased to 3.6 and 3.3 mm when we added 250 mg / Disk of harmala extract to medium for each bacteria . There is no clear increase in the inhibition zone untile we used concentration at 500 mg / Disk . Gram positive bacteria , *S. aureus* and *Bacillus subtilis* were showed highly sensitive to the concentrations ( 125 , 250 and 500 mg / Disk) of harmala extracts , which caused clear increased in the inhibition zone for each bacteria , the range of the inhibition zone for *S.aureus* increased from 4.7 for 125 mg/ disk to 14.0 mm for 250 mg / Disk , while for *B. subtilis* . the range varied from 3.3 for 125 mg / Disk to 11.2 mm for 500 mg / Disk . from these Results , the gram positive bacteria were highly sensitive to harmala at various concentrations comparsion with gram negative bacteria . Similar to the results obtained in this report , Musa and Mohammd (1992) were found that the inhibition percentage was very high for *S. aureus* when 200 mg of harmala extract were added to medium , while *E.coli* needed to 400 mg to inhibited the growth , also they were found that hormala extract caused inhibition for the microbial**

**Table ( 1 ) . Effect of harmala extract on the growth of gram positive and negative bacteria in solid medium .**

| harmala extract concentrations mg/disk | diameter of inhibition zone ( mm ) | | | |
|---|---|---|---|---|
| | *S.aureus* | *B.subtilis* | *E.coli* | *Ps.flourescens* |
| 0 | 0 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| 125 | 4.7 | 3.3 | 2.1 | 2.0 |
| 250 | 9.4 | 8.1 | 3.6 | 3.3 |
| 500 | 14.0 | 11.2 | 4.7 | 4.9 |

content of minced beef . On the other hand , the effect of some spices , herbs and vegetable extracts were studied and similar findings has been reported by other investigators ( Shelef *et al* ., 1980 ; El-Kady *et al* ., 1993 ; Mohmmd , 1995 , 2005 ) .

These articles showed that the sensitivity of gram positive bacteria maybe due to the absence of lipopolysaccharide in cell wall which prevent the effectiveness of these antibacterial agents . (Musa and Mohmmd, 1995; Anthony, 1976 ) . *S.aureus* was more sensitive to various concentrations of harmala extracts . The effect of harmala on *S. aureus* growth in liquid medium was studied in detial , table (2) . During 6 days of incubation at 37 C harmala extract at concentration 200 ppm reduced the growth of S. aureus from 5.31 CFU / ml for control ( bacterial growth without harmala ) to 3.61 CFU/ ml after 6 days of incubation at 37 C , while there was high decrease in the growth of *S. aureus* occurred at 600 ppm of harmala extract , which caused clear reduction after 6 days of incubation at 37 C , The log No. of *S. aureus* reduce to 2.01 CFU/ ml . The antibacterial activities in harmala extract against these bacteria may be due to the major antibacterial compounds , which were classified by other researchers (Hussain , 1981 ; Musa and Mohmmd , 1992) .

Table ( 2 ). Growth of *S. aureus* in Nutrient both containing
200 and 400 ppm of harmala extract .

| Incubation period at 37C ( days ) | Log CFU / ml | | |
|---|---|---|---|
| | 0 | 200 ppm | 400 ppm |
| 0 | 5.31 | 5.31 | 5.31 |
| 2 | 6.22 | 3.61 | 2.71 |
| 4 | 8.10 | 3.50 | 2.42 |
| 6 | 7.15 | 2.17 | 2.01 |

**Antifungal activities :**

The effect of various levels of harmala extracts on mycelium growth and T-2 toxin was shown in Table (3) . The concentration 200 ppm of harmala extracts caused no effect on Fusarium growth and T-2 toxin production while the coincentration 400 ppm caused high decrease in mycelial growth as well as low a decrease in $T-_2$ toxin . The extent of the decrease varied with the concentration of harmala extract . Maximum decrease ( 1.33 ug/50 ml ) in mycelial growth was observed with the concentration of 400 ppm followed( 96.5 ug/ml ) T-2 toxin production at 600 ppm . The decrease in T-2 toxin production was accompained by an increase in mycelial growth for *Fusarium acuminatum* at concentration 600 ppm of harmala extract .

The addition of harmala extract to liquid medium caused a decrease in mycelial mass and enhanced T-2 toxin production . The inhibitory effects of harmala on the growth of *Fusarium acuminatum* and $T-_2$ toxin may due to the inhibition of fungal growth or metabolic precursor pool for T-2 toxin production or both ( Tiwar *et al* ., 1980 ). The results

**obtained from this study agreed with some reports that show high effective for preservatives or sorbates ( Garies *et al* ., 1984 ; Mohmmd , 2006 ) and some spice essential oils on some fungi and production of mycotoxins ( Farag and –abo-raya , 1989 ; al-Kady *et al* ; 1983 ; Mohmmd , 2004 , 2005). little is known about the effect of herbs , drugs and spices on the growth of fungi and mycotoxin production , while no report is available concerning the influence of compound that found in medical plants on T-2 toxin production .**

**In conclusion we need to study the chemical nature of the compounds in harmala which caused inhibitory effect on the bacterial growth or mycelium mass and production of T-2 toxin .**

**Table (3) : Effect oh harmala extract in liquid medium on Mycelium growth and T-2 toxin production by *Fusarium acuminatum* incubated at 25 C for 20 days .**

| Harmala extract concentration (ppm) | Dry weight of mycelium g / 50 ml | T-2 toxin concentrations ug/ml |
|---|---|---|
| 0 | 1.90 | 119.5 |
| 200 | 1.81 | 118.3 |
| 400 | 1.33 | 116.3 |
| 600 | 1.60 | 96.5 |

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**دراسة تأثير مستخلصات الحرمل *Peganum harmala* على نمو بعض البكتريا والفطر *Fusarium acuminatum* المنتج للسم T-2**

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**الخلاصـــــة :**

**تم دراسة تأثير تراكيز مختلفة لمستخلصات بذور نبات الحرمل *Peganum harmala* على نمو أربع عزلات من البكتريا وعزلة للفطر *Fusarium acuminatum* المنتج للسم T-2 . اظهرت نتائـــــج الدراسة الحساسية العالية للبكتريا الموجبة لصبغة كـــــرام ( *Staphylococcus aureus*و *Bacillus subtilis* ) لكافة التراكيز المستخدمة ( 125 - 500 mg / Disk ) من مستخلصات الحرمل مقارنة بالبكتريا السالبة لهذه الصبغة ( *Escherichia coli* و *Pseudomonas fluorescens* ) وكانت بكتريا *S.aureus* الاكثر حساسية من بين الانواع الاخرى . كما أشارت نتائج الدراسة الى ان مستخلصات الحرمل المستخدمة كانت ذو فعالية عالية جدا في التأثير على نمو الفطر من خلال اختزال وزن المايسليم الجاف من 1.90 الى 1.33 مايكروغرام / 50 مل ، وفي اختزال كمية السم T-2 المنتج من الفطر في الوسط الغذائي السائل ( كان الانخفاض للسم من 119.1 الى 96.5 مايكروغرام / مل ) عند تركيز 600 PPM بعد 20 يوم من الحضن على درجة حرارة 25 م .**