Effect of *Peganum harmala* Seeds on Blood Factors, Immune Response and Intestinal Selected Bacterial Population in Broiler Chickens

Majid Goudarzi

***Abstract*—** This experiment was designed to study the effects of feeding different levels of *Peganum harmala* seeds (PHS) and antibiotic on serum biochemical parameters, immune response and intestinal microflora composition in Ross broiler chickens. A total of 240 one-d-old unsexed broiler chickens were randomly allocated to each of the 4 treatment groups, each with 4 replicate pens of 15 chicks. The dietary treatments included of control (C) - without PHS and antibiotic - the diet contains 300 mg/Kg Lincomycin 0.88% (A) and the diets contain 2g/Kg (H1) and 4g/Kg (H2) PHS. The chicks were raised on floor pens and received diets and water ad libitum for 6 weeks. Blood samplings were performed for determine of antibody titer against Newcastle disease on 14 and 21 days and for biochemical parameters on 42 days of age. The populations of Lactobacilli spp. and Escherichia coli were enumerated in ileum by conventional microbiological techniques using selective agar media. Inclusion of PHS in diet resulted in a significant decrease in total cholesterol and significant increase in HDL relative to the control and antibiotic groups. Antibody titer against NDV was not affected by experimental treatments. The *E. coli* population in birds supplemented with antibiotic and PHS was significantly lower than control but the *Lactobacilli spp.* population increased only by antibiotic and not by PHS. In conclusion, the results of this study showed that addition of *Peganum harmala* seeds powder seem to have had a positive influence on some biochemical parameters and gastrointestinal microflora.

.

***Keywords*—** Antibiotic, Biochemical parameters, Immune system, *Peganum harmala*.

I. Introduction

Growth promoters such as antibiotics play an active role in commercial production of poultry. Although, good results are obtained with these substances, their use might have unfavorable effects, in other side it may also result in production of residual problems in the tissues of birds and animals [1]. For this reason, most antibacterial performance promoters recently have been banned in many countries. It is indispensable to minimize these components, and deals with replacers without any adverse effect on production. The medicinal plants have been used traditionally in the therapy of some diseases in human for a long time. Also in recent years the effects of medicinal herbs have been studied on various indices such as performance, immunity and serum biochemical profiles in broilers [2], [3], [4]. In practice, herbs, their extracts and the components extracted from them have been used as an alternative for antibiotic growth promoters [5], [6].

Peganum harmala - known as "Espand" in Iran- belongs to family Zygophylaceae is a traditional medicine. It has been employed for the treatment of a range of human diseases [7]. This herb contains several the beta carboline alkaloids such as harmaline, harmine, harmalol and harmol. They have some of pharmacological and biological activities such as antibacterial and antifungal [8], anticoccidial [9], disinfectant [10], growth promoting [11], cholesterol lowering and hepatoprotective effects [12], Glucose lowering [13], monoamineoxidase inhibition [14], hypothermic [15], platelet aggregation inhibitory [16], immunomodulatory effects [17] and anti-inflammatory [18].

Reference [19] announced that the extract of *Peganum harmala* has limited antimicrobial activity against E. coli in vivo. Reference [9] concluded that P. harmala has the anticoccidial effect in broiler chicks. Reference [20] reported that the blood HDL and LDL concentration increased and decreased, respectively, by using of methanolic extract of Harmal in diet. In spite of these findings, there has been a dearth of information on the effect of harmala on, serum biochemical parameters, and intestinal microflora in comparison with an antibiotic growth promoter in broiler chickens. Therefore, the present study was design to compare and survey the effect of two levels of P. harmala and Lincomycin antibiotic onance, serum biochemical parameters, and intestinal microflora in broiler chicks.

II. Materials and Methods

A. Animals and diets

A total of 240 one-d-old unsexed broiler chickens (mean initial weight: 37.5 ± 1 g) were randomly allocated to each of the 4 treatment groups, each with 4 replicate pens of 15 chicks. The dietary treatments included of control (C) -

Majid Goudarzi is with Department of Animal Science, College of Agriculture, Boroujerd Branch, Islamic Azad University, Boroujerd, Iran (majidgoudarzi117@gmail.com).

without *Peganum harmala* seeds (PHS) and antibiotic - the diet contains 300 mg/Kg Lincomycin 0.88% (A) and the diets contain 2g/Kg (H1) and 4g/Kg (H2) PHS. The basal diet (Table I) was formulated according to the nutrient requirements (NRC 1994) based on corn and soybean meal: starter (1 to 21 d) and grower (22 to 42 d). *Peganum harmala* seed powder and antibiotic were added on top of the basal diets. The *P. harmala* were supplied from a local market and the dry seeds were cleaned of foreign materials and seeds and milled to a soft powder. The chicks were raised on floor pens (0.096m2/bird) for 6 weeks and during this period, they received diets and water ad libitum. The bird had access to feed and water through a tube feeder and a manual waterer in each pen. The chicks were reared under a lighting program which included of 23 hours of light and 1 hour of darkness. The ambient temperature was 32oC ± 1 during the first week, and it was reduced three degrees per week in following weeks and finally it was maintained at 22oC.

B. Serum Biochemistry

At the end of period after 8 hours of starvation, the birds were killed by serving the jugular vein and carotid artery on one side of the neck and allowed to bleed. The blood samples were collected from 8 birds in each treatment to assess of biochemical parameters. The blood was centrifuged at 2000 ×g for 15 min to obtain serum (SIGMA 4 - 15 Lab Centrifuge, Germany). Individual serum samples were analyzed for total protein, total cholesterol, high-density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (Pars-Azmoon Co., Tehran).

C. Antibody Titer

The birds were vaccinated against Newcastle disease virus (NDV) subcutaneously with 0.2 mL per bird at 7 days of age. The blood samples were collected from the brachial vein of two randomly selected birds of each replicate at 14 and 21 days of age. The samples were centrifuged at 2000 ×g for 15 min to obtain serum (SIGMA 4 - 15 Lab Centrifuge, Germany). Antibody titers against NDV were measured by hemagglutination Inhibition Test according to procedure described by [21].

D. Enumeration of bacteria populations in ileum

For a determination of some selected micro-organisms in intestinal digesta from 32 birds (2 birds per replication) the digesta samples were collected from ileum and used for microbial assays within 1 h from collection. Digesta samples were serially diluted in 0.85% sterile saline solution for enumeration of *Lactobacilli spp.* and *Escherichia coli* (E. coli) by conventional microbiological techniques using selective agar media. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. *Lactobacilli spp.* was anaerobically assayed using MRS agar (Fluka 80961). Lactobacilli spp. was confirmed by using API 50 CH kit (Biomerieux_ SA, Marcy-l'Etoile/France). *E. coli* were enumerated through the use of Plate Count MUG Agar (Fluka 80961) and TBX Agar (Fluka 92435). Results were expressed as base-10 logarithm colony-forming units per gram of digesta.

E. Statistical Analysis

All data were analyzed by one-way ANOVA using the GLM procedure of SAS for Windows version 9.1 [22]. The data were analyzed base on following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

where Y_{ij} is the dependent variable, μ is the general mean, T_i is the treatment effect of the i^{th} treatment, and ε_{ij} is the random error. The significance of differences between means was compared by using of the Duncan's multiple range tests of SAS. Significance was declared at $P \leq 0.05$ for all variables measured.

TABLE I.
THE INGREDIENT AND CALCULATED COMPOSITION OF BASAL DIETS

Item	Starter (0-21)	Grower (22-42)
Ingredient, g/kg		
Corn	543.5	656.3
Soybean meal (43.8%)	378.1	292.8
Soybean oil	40.0	17.5
Di calcium phosphate	12.9	10.9
Oyster sell	14.1	13.2
NaCl	4.4	3.1
Mineral premix1	2.5	2.5
Vitamin premix2	2.5	2.5
DL-Methionine	1.4	0.6
Lysine	0.6	0.6
Analysis results		
Metabolizable energy (kcal/kg)	3000	3000
Crude protein (g/kg)	215.7	187.6
Calcium (g/kg)	9.4	8.4
Available phosphorus (g/kg)	3.8	3.3
Methionine(g/kg)	4.9	3.7
Lysine(g/kg)	12.0	1.0
Methionine + Cysteine (g/kg)	8.4	6.8

1-Ingredients per kg: Mg, 60 g; Fe, 80 g; Cu, 10 g; Zn, 50 g; Co, 2 g; I, 1 g,
2- Ingredients per kg : vitamin A, 1000,000 IU; D3, 1500000 IU; E, 15000 IU; K, 3g; B1 2g; B2, 4 g; B6, 3g; B12, 0.015 g; pantothenic acid, 10 g; nicotinic acid, 2 g; folic acid, 1 g; choline, 250g ; Se, 100 g

III. RESULTS AND DISCUSSIONTH

Inclusion of 4 and 2 g/Kg PHS in diet resulted in a significant decrease in total cholesterol relative to the control and antibiotic groups (Table II). The HDL was increased by using 2 g/Kg PHS in diet. The other parameters include triglyceride, LDL and total protein were not affected by dietary treatments.

Reference [20] found that 250 mgL-1 methanolic extract of *Peganum harmala L.* in drinking water reduced total cholesterol, triglycerides and LDL cholesterol and increased HDL cholesterol in broiler chicks. Reference [12] reported similar results in male Wistar Rat. Reference [23] found that the concentration serum cholesterol decreased in the chicks fed a diet containing 10% *Peganum harmala L.* leaves.

Reference [24] fed 2.5 kg P. harmala seeds and 2.5 kg chamomile flower heads per ton of broiler diet and observed reduced serum cholesterol as compared to the control group. In contrast to these findings**,** Reference [25] did not observe significant difference in total cholesterol and HDL by using aqueous and ethanolic extract of Harmala seeds. The variation in findings of reducing cholesterol is probably due to the difference in experimental designs, ages and species of animal and types and parts of the plant used. The key enzyme involved in regulating cholesterol metabolism is 3-hydroxy-3methyl-glutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway. By competitively blocking this enzyme, the HMG-CoA reductase inhibitors interfere with cholesterol formation. As a result, they decrease total cholesterol, LDL, apolipoprotein B (a membrane transport complex for LDL-C), very low-density lipoprotein (VLDL), and plasma triglycerides. They also increase serum concentrations of HDL [26]. It is possible that the HMGCoA reductase has been inhibited by different alkaloids harmine, harmaline, harmol, present in *P. harmala*. This plant has antioxidant property [27 which reduce LDL oxidation and thereby reducing total cholesterol content. Reference [28] reported that the harmaline had a markedly higher antioxidant capacity than harmine in scavenging or preventive capacity against free radicals as well as inhibiting the aggregation of the LDL protein moiety (apolipoprotein B) induced by oxidation.

TABLE II
EFFECT OF EXPERIMENTAL DIETS ON BIOCHEMICAL PARAMETERS

Variable	Treatments(mg/dl)				
	C	A	H1	H2	SEM
Triglyceride	*69.50*	*74.00*	*80.50*	*85.00*	*3.31*
Total Cholesterol	*119.30ab*	*125.50a*	*108.00b*	*113.25b*	*2.32*
LDL - Cholesterol	*15.00*	*13.75*	*14.00*	*13.75*	*0.69*
HDL - Cholesterol	*84.75ab*	*82.00b*	*91.00a*	*86.75ab*	*1.46*
Total protein	*3.20*	*3.25*	*3.18*	*3.20*	*0.05*

*Values in the same row not sharing a common superscript differ significantly (P≤0.05). SEM = Standard error of mean

As shown in Table III the using of antibiotic and PHS in diet failed to induce any significant effects on antibody titers against NDV at 14 and 21 days of age (P > 0.05).

Result of this trial showed that, humoral immune responses were not affected by dietary treatments. As harmala has been reported to have antimicrobial [8] and antioxidant activities [9], an increase in immune responses of chicks was anticipated. Similar results have been reported in some previous researches [29], [3] with other herbs. Unfortunately, there are a few reports on the effects of harmala on bird immune responses. The result of this study is in contrast to the results of [30] who reported that the methanolic extract of *P. harmala* significantly improved antibody titer against ND at day 21 and 28 when used at the rate of 250 mg/L of drinking water. Reference [17] reported that *P. harmala* extract with optimal dose of 100 mg/kg can act as immunostimulants and enhance the immune response of cultured fish.

TABLE III
EFFECT OF EXPERIMENTAL DIETS ON ANTIBODY TITERS AGAINST NDV AT 14TH AND 21ST DAYS

Antibody titers (log10)	Dietary treatments				
	C	A	H1	H2	SEM
14 days	0.696	0.750	0.706	0.737	0.014
21 days	0.812	0.817	0.807	0.828	0.013

SEM = Standard error of mean

Data on ileum bacteria populations of broiler chicks at Day 42 of age (table IV) showed that the using antibiotic and PHS in diet had significant (P<0.05) effect on *E. coli* and *Lactobacilli spp*. populations. The *E. coli* population in birds supplemented with antibiotic and PHS was significantly lower than control. The effect of antibiotic on it was bigger than PHS. The difference between H1 and H2 groups were not significant. The *Lactobacilli spp.* population increased only by antibiotic and not by PHS. The differences between control and PHS groups were not significant (P>0.05).

TABLE IV
EFFECTS OF DIETARY TREATMENTS ON ILEUM BACTERIA POPULATIONS OF BROILER CHICKS AT D 42 OF AGE.

Variable	Dietary treatments				
	C	A	H1	H2	SEM
E. coli	6.875a*	5.850c	6.425b	6.325b	0.102
Lactobacilli spp.	4.675b	5.225a	4.700b	4.850b	0.082

*Values in the same row not sharing a common superscript differ significantly (P≤0.05).
SEM = Standard error of mean

It has been reported that the *Peganum harmala* has antibacterial, antifungal and antiviral effects [31]. It traditionally has been used as an antiseptic and disinfectant agent by burning its seeds in Iran [19]. An ethanolic *P. harmala* extract has been shown to have high antibacterial activity against *MRSA* (methicillin resistant *Staphylococcus aureus*) [32] and *CRSA* (cefixime resistant *S. aureus*) [33]. References [3] and [8] showed inhibitory effect of alcoholic extract of *P. harmala* seeds on the growth of *E. coli*. Reference [19] announced that the extract of *Peganum harmala* has limited antimicrobial activity against *E. coli* in vivo, but long-term continuous feeding may induce undesirable effects. Reference [35] reported that the β–carboline alkaloid isolated from the aerial parts of *Peganum harmala* showed significant antibacterial activity against *Streptococcus pyogenus*. It has been reported that harmane as a highly aromatic planar alkaloid exerts its antibacterial activity through interchalate with DNA [36], thus, this antibacterial mechanism must be considered for active extract of *P. harmala*.

IV. CONCLUSION

The results of this study showed that *Peganum harmala* seeds have positive effect on some serum biochemical parameters and ileum bacteria populations in broiler chicks. The adding of *Peganum harmala* seeds in diet resulted in

decreasing of in total cholesterol and increasing of HDL. Also, the using PHS in diet led to the lower *E. coli* population in ileum. There is not any significant different between 2 and 4 g/Kg levels of PHS.

ACKNOWLEDGMENT

The authors wish to express special thanks for salaries and research support by Islamic Azad University, Borujerd branch.

REFERENCES

[1] Burgat, "Residues of drugs of veterinary use in food," La Revue du Praticien, vol.41, no. 11, pp. 985-990, May 1991.

[2] SH. Nanekarani, M. Goodarzi, M. Heidari, N. Landy, "Efficiency of ethanolic extract of peppermint (Mentha piperita) as an antibiotic growth promoter substitution on performance, and carcass characteristics in broiler chickens," *Asian Pacific Journal of Tropical Biomedicine* S1611-S1614, Dec, 2012.

[3] M. Goodarzi, N. Landy, Sh. Nanekarani, "Effect of onion (Allium cepa L.) as an antibiotic growth promoter substitution on performance , immune responses and serum biochemical parameters in broiler chicks," *Health*, vol. 5, no. 8, pp. 1210-1215, July 2013.

[4] M. Goodarzi, N. Mohtashami Pour, D. Modiri, **"**The effect of savory (*Satureja khuzistanica*) essential oils on performance and some blood biochemical barameters of Ross and Cobb broilers," *Annual Research &Review in Biology*, vol. 4, no. 24, pp. 4336-4343, Aug. 2014.

[5] N. Ocak, G. Erener, F. Burak Ak, M. Sungu, A. Altop, A. Ozmen, "Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Tymus vulgaris* L.) leaves as growth promoter source," *Journal of Animal Science*, vol. 53 no. 4, pp.169-175, Apr. 2008.

[6] T. Ayasan, "Effects of dietary Yucca schidigera on hatchability of Japanese Quails," *Indian Journal of Animal Sci*ence vol. 83, no. 6, pp. 641-644. June 2013.

[7] N. Aslam, A. A. Wani, I.A. Nawchoo, M.A. Bhat, "Distribution and Medicinal importance of *Peganum harmala*- A review," *International Journal of Advanced Research* vol. 2, no. 2, pp.751-755, Feb. 2014.

[8] E. Darabpour, H. Motamedi, A. Poshtkouhian Bavi, S.M. Seyyed Nejad, "Antibacterial activity of different parts of *Peganum harmala L*. growing in Iran against multi-drug resistant bacteria," *EXCLI Journal*, vol.10, pp. 252-263. Nov. 2011.

[9] A.J. Tanweer, N. Chand, U. Saddique, C.A. Bailey, R.U. Khan, " Antiparasitic effect of wild rue (*Peganum harmala L.*) against experimentally induced coccidiosis in broiler chicks," *Parasitology Research*, vol. 113, pp. 2951-2960, May 2014.

[10] A.R. Shahverdi, H.R. Monsef-Esfahani, B. Nickavar, L. Bitarafan, S. Khodaee, N. Khoshakhlagh, "Antimicrobial activity and main chemical composition of two smoke condensates from *Peganum harmala* seeds," *Zeitschrift für Naturforschung C. A Journal of Biosciences*, vol. 60, no. 10, pp. 707–710, Oct. 2005.

[11] A.J. Tanweer, N. Chand, S. Khan, M.S. Qureshi, A. Akhtar, M. Niamatullah, "Impact of methanolic extract of *Peganum Harmala* on the weight gain, feed conversion ratio, feed cost and gross return of broiler chicks," *The Journal of Animal & Plant Sciences*, vol. 22, no. 2, pp. 264-267, 2012.

[12] A.m. Eini, H. Fazaely, M. Moharrami Fard, M. Meini, M. Drab M. Ahmadi Far, "Effect of *Peganum harmala L*. on lipid parameters in hypercholesterolemia-induced male Wistar Rat," *Academia Journal of Medicinal Plants*, vol. 2, no.5, pp. 074-078, Aug. 2014.

[13] A.B. Singh, J.P. Chaturvedi, T. Narender, A.K. Srivastava, "Preliminary studies on the hypoglycemic effect of *Peganum harmala L*. Seeds ethanol extract on normal and streptozotocin induced diabetic rats," *Indian Journal of Clinical Biochemistry*, vol. 23, no. 4, pp. 391-393, 2008.

[14] E. Salari, K. Ahmadi, R.Z. Dehyaghobi, A. Purhematy H.M. Takalloozadeh, "Toxic and repellent effect of harmal (*Peganum harmala L*.) acetonic extract on several aphids and Tribolium castaneum," *Chilean Journal of Agricultural Research*, vol. 72, no.1, pp. 147-151, Mar. 2012.

[15] A.F. Abdel-Fattah, K. Matsumoto, H.A. Gammaz, H. Watanabe, "Hypothermic effect of harmala alkaloid in rats: involvement of serotonergic mechanism," *Pharmacology Biochemistry & Behavior*, vol. 52, no. 2, pp. 421–426, Oct. 1995.

[16] S.A. Saeed, S. Farnaz, R.V. Simjee, A. Malik, "Triterpenes and β-sitosterol from piper betel: Isolation antiplatlet and anti-inflammatory effects," .*Biochem soc Trans*, vol. 21, no. 4, pp. 462s. Nov. 1993.

[17] M. Ghareghani Poor, P. Akbary, M. Akhlaghi, M.S. Fereidouni, "Non specific immune response of rainbow trout (oncorhynchus mykiss, walbaum) fed with seed extract of *peganum harmala L*., *Indian Journal of Fundamental and Applied Life Sciences*, vol. 4, no. 3, pp. 249-255, Sep. 2014.

[18] H.R. Monsef, A. Ghobadi, M. Iranshahi, M. Abdollah, "Antinociceptive effects of *Peganum harmala L*. alkaloid extract on mouse formalin test," *Journal of Pharmaceutical Sciences*, vol. 7, no. 1, pp. 221-222, Feb. 2004.

[19] N. Arshad, K. Zitterl-Eglseer, S. Hasnain, M. Hess, "Effect of *Peganum harmala* or its beta-carboline alkaloids on certain antibiotic resistant strains of bacteria and protozoa from poultry," *Phytotherapy Research*, vol. 22, no. 11, pp. 1533-1538, Nov. 2008.

[20] A.J. Tanweer, N. Chand, S. Khan, S. Sultan, M.S. Qureshi, A. Akhtar, M. Inam, "Association of *Peganum harmala L.* supplementation with lipid profile and its economic benefit in broiler production," *Pak Vet J.*, vol. 33, no. 3, pp. 313-316, Feb. 2013.

[21] S.G. Thayer, C.W. Beard, "Serologic proce- dures. In: Swayne, D.E., Ed., *A Laboratory* Manual for the Isolation and Identification of Avian Pathogens," 4th Edition, American Association of Avian Pathologists, Phi- ladelphia, pp. 256-258, 1998.

[22] SAS. "User's Guide, Statistics," SAS Institute, Cary, NC. Schmidt, E. and F.W. 1998.

[23] W.S. Qazan, "The effect of low levels of dietary *Peganum harmala L.* and *Ballota undulata* or their mixture on chicks," *Journal of Animal and Veterinary Advances*, vol. 8, no. 8, pp. 1535-1538, 2009.

[24] I.M., Abaza, M.A. Asar, G.E. Elshaarrawi, M.F. Hassan, "Effect of using Nigella seeds, Chamomile flowers, Thyme flowers and Harmala seeds as feed additives on performance of broiler," *Egypt. J. of Agric. Res.*, Vol. 81 no. 2, pp. 735-750, 2003.

[25] M.M. Hasanzadeh Tahery, M. Hassanpour Fard, N. Rabiee, A. Ghorishe, N. Ravanbakhsh, "Effects of aqueous and ethanolic extracts of *Peganum harmala L.* seeds on lipids profile in rats," *Journal of Birjand University of Medical Sciences*, vol. 20, no. 2, pp. 108-114. Aug. 2013.

[26] M. Ciftci, U.G. Simsek, A. Yuce, O. Yilmaz, B. Dalkilic, "Effects of dietary antibiotic and cinnamon oil supplementation on antioxidant enzyme activities, cholesterol levels and fatty acid compositions of serum and meat in broiler chickens," *Acta Vet. Brno*, vol. 79, no. 1, pp. 33–40, Sep. 2010.

[27] A. Jinous, R. Fereshteh, "Chemistry, pharmacology and medicinal properties of *Peganum harmala L*.," *Afr. J. Pharm. Pharmacol*, vol. 6, no. 22, pp. 1573-1580, June 2012.

[28] H. Berrougui, M. Isabelle, M. Cloutier, M. Hmamouchi, A. Khalil, "Protective effects of *Peganum harmala L*. extract, harmine and harmaline against human low–desity lipoprotein oxidation," *J. Pharmacy & Pharamacol*, vol. 58, no. 7, pp, 967-974, July 2006.

[29] S. Rahimi, Z. Teymouri Zadeh, M.A. Karimi Torshizi, R. Omidbaigi H. Rokni, "Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens," *Journal of Agricultural Science and Technology*, vol. 13, no. 4, pp. 527-539, Aug. 2011.

[30] A.J. Tanweer, N. Chand, S. Khan, M.S. Qureshi, U. Sadique, A.U. Rehman, A. Sultan, M. Arshad, A. Akhtar, S. Jan "Association of *peganum harmala l*. Supplementation with immunity against ND, IB and IBD in broiler chicks," *Pakistan Journal of Science*, vol. 66, no. 1, pp. 88-94, Mar. 2014.

[31] M. Shonouda, S. Osman, O. Salama, A. Ayoub, "Toxical effect of *Peganum harmala L.* leaves on the cotton leaf worm, Spodoptera littoralis Boised and its parasitoids Microlitis refiventris Kok," *Pak J Biol Sci*, vol. 11, no 4, pp. 546-552, 2008.

[32] M.S. Moghadam, S. Maleki, E. Darabpour, H. Motamedi, S.M. Seyyednejad, "Antibacterial activity of eight local plant extracts in Khouzestan, Iran against methicillin and cefixime resistant Staphylococcous aureus strains," *Asian pacific J Trop Med*, vol. 3, no. 4, pp. 262- 265, Apr. 2010.

[33] D. Prashanth, S. John, "Antibacterial activity of *Peganum harmala*," *Fitoterapia*, Vol. 70, no. 4, pp. 438-439, Aug. 1999.

[34] MM. Mashreghi, S. Niknia, "The effect of *Peganum harmala* and *Teucrium polium* alcoholic extracts on growth of Escherichia coli O157," *Jundishapur Journal of Microbiology*, vol. 5, no. 3, pp. 511-515, Dec. 2012.
[35] H.G. Abdel Aziz, S.M. Abdel Kader, M.M. El-Sayed, E.A., EL-Malt, E.S. Shaker, "Novel β–Carboline alkaloidfrom *Peganum Harmala* as antibacterial agant," in proc. *Tenth Radiation Physics & Protection Conference*, Nasr City - Cairo, Egypt, 27-30 November 2010.
[36] M.M. Cowan, "Plant products as antimicrobial agents," *Clin. Microbiol Rev*, vol. 12, no.4, pp. 564- 582, Oct.1999.