Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparison between Effect of Chemical and Biological Fertilizers on Yield and Yield Components Wheat (*Triticum aestivum* L.)Pishtaz Cultivar

Amin Farnia[1]* and Kurosh Hasanpoor[2]

Assistant Professor of Department of Agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.
M.Sc student of Department of Agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.

Received: 20 Mar 2015 Revised: 11 Apr 2015 Accepted: 28 May 2015

***Address for correspondence**
Amin Farnia,
Assistant Professor of Department of Agriculture,
Boroujerd branch,Islamic Azad University,
Boroujerd, Iran.
E-mail: Aminfarnia@yahoo.com

ABSTRACT

A field experiment was laid out in order to study on comparison between effect of chemical and Biological fertilizers on yield and yield components wheat (*Triticum aestivum* L.) Pishtaz cultivar. The experiment was laid out in a factorial design based on randomized block design with three replications. Treatments were chemical fertilizers in two levels (Urea and Ammonium nitrate) and biological fertilizers in three levels (4L.ha^{-1}Nitroxin, 4L.ha^{1} Nitroplus and 200g.ha^{-1}Nitrokara) with control for them. The results of this study showed that yield and yield components of wheat affected by chemical and biological fertilizers significantly Application of chemical and biological fertilizers increased the average number of spikelet per spike, number of grin per spike, 1000 grain weight, biomass and grain yield and HI rather than non-application of them. For chemical fertilizers maximum plant height, number of grains per spike, 1000 grain weight and grain yield was recorded at application of Urea treatment. However, for biological treatment maximum number of spikelet per spike was recorded at application of Nitroxinbiofertilizer. Interaction effect of chemical and biological fertilizers shoed that application of Urea and Amunium with Nitroplus and Nitrokara had the highest biomass yield.Also, control treatment had the highest harvest index. In final our results showed that application of chemical and biological fertilizers increased yield and yield components of wheat but chemical fertilizers specially Urea fertilizer increased them more than biological fertilizers.

Key words: Chemical fertilizer, wheat, and Yield components, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop in Iran. Increasing wheat production is an essential national target to fill the gap between production and consumption. Wheat cultivars production is one of the most important factors which play a major role in increases wheat production and contribute in food problem solves (10).The world does not have enough potential for increasing the soil level cultivated with wheat; therefore in order to increase the wheat production, we have to increase the productivity of the fields which have been cultivated with wheat. Grain yield of small grain cereals is determined by two main components, grain number per unit area (grains perm^2) and mean grain weight. Environmental conditions around 20 days pre- and 10 days post-anthesis are considered critical for grain yield determination (16). According to the statistics of the food and agriculture organization (FAO), during 2008-2009 growing season 682 million tons of wheat were produced and it is estimated that up to 690 million tons will be produced in 2012- 2013 growing season.

Organic farming has emerged as an importantpriority area globally in view of thegrowing demand for safe and healthy foodand long term sustainability and concernson environmental pollution associated withindiscriminate use of agrochemicals (2).Though the use of chemical fertilizers inagriculture is inevitable to meet thegrowing demand for food in world, thereare opportunities in selected crops andniche areas where organic production canbe encouraged to tape the domestic exportmarket (11). Biofertilizers are important not only for thereduction in quantity of chemical fertilizersbut also for getting better yield insustainable agriculture(2). Organic agricultureis a holistic production management system which promotes and enhances agroecosystem, health, including biodiversity,biological cycles, and soil biologicalactivity (2).Bio-fertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms (Goelet al, 1999) providing a more balanced nutrition for plants (6). El-Ghadbanet al (8) found that fennel responded to biofertilizer by increasing growth and oil yield and changing the chemical composition.

Azimi et al (4) found that that application nitrogen and phosphate biofertilizers increased yield and yield components of barley under Boroujerd environmental condition. They suggested that Grain yield and biomass yield increasing was reported with the biofertilizer application which account important benefit, causing decreasing in the inputs of production because of economizing much money to chemical fertilizers and increasing in yield and biological yield. Increasing yield was attributed to the plant growth promoting substances by root colonizing bacteria more than the biological nitrogen fixation, (13) stated that yield increased due to promoting root growth which in turn enhancing nutrients and water uptake from the soil. Beyranvand et al (7) reviled that application nitrogen and phosphate biofertilizers increased yield and yield components of maize under Boroujerd environmental condition. They suggested that effect of nitrogen and phosphate biofertilizers were evaluated positively, there were an increase in plant height, ear weight, number of grain per cob, grain yield and biomass yield.

Therefore the aim of this study is comparison between effect of cemical and biological fertilizers on yield and yield components of common wheat in Dorud, Lorestanprovienve, Iran.

MATERIAL AND METHODS

Field material and Experimental design

This field experiment was laid out in the Faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch, Boroujerd, Iran during the growing seasons 2013- 2014. The experiment was laid out in order to evaluate the comparison between effect of chemical and biological fertilizers on yield and its components of common

wheatPishsaz cultivar. The Boroujerd region has a continental semi-arid climate with annual precipitation of 369 mm. About 50% of this falls during the wheat and barley growing period.

Treatments

The experiment was laid out in a factorial design based on randomized block design with three replications. Treatments were chemical fertilizers in two levels (Urea and Ammonium nitrate) and biological fertilizer in three levels (4l.ha^{-1}Nitroxin, 4l.ha^{1} Nitroplus and 200g.ha^{-1}Nitrokara) with control for them.

Yield and yield components determination

For sowing, 200kg.ha^{-1}seed was used. There were 4 rows in each from 36 plots; rows were 5 m long with 0.25 m row spacing. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle 4 m^2 of the two central rowswere harvested.Then yield components were calculated as standard methods with using 10 plants. To determine grain yield, biomass yield and harvest index, we removed and cleaned all the seeds produced within two central rows in the field. Then grain yield and biomass yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter andquintals per hectare. Replicated samples of clean seed (broken grain and foreign materialremoved) were sampled randomly and 1000-grain were counted and weighed. Theharvest index was accounted with follow:

HI = (Economical yield / Biological yield)*100

Statistical analysis

The statistical analyses to determine the individual and interactive effects of treatments were conducted using JMP 5.0.1.2 (15). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effectsfrom the two runs of experiments followed a similar trend, and thus the data from the twoindependent runs were combined in the analysis.

RESULTS AND DISCUSSION

Plant height :Analysis of variance of results showed that, the effect of chemical fertilizer on plant height was significant only (table 1). The comparison of the mean values showed that application of Urea had the highest (96cm) and the control treatment had the lowest (82cm) plant height and difference between them was significant (figure 1).

Number of spikelet per spike: The results showed that, the effect of biological fertilizer on number of spikelet per spike was significant (table 1).The comparison of the mean values of the number of spikelet per spike showed that application ofNitroxin has the highest (27) and controltreatment had the lowest number of spikelet per spike (17) and difference between them were significant (figure 2).

Number of grain per spike :The effect of chemical fertilizer on number of grains per spike was significant only (table 1). The comparison of the mean values for number of grain per spike for wheat showed that application of Urea had the highest (28) and the control treatment had the lowest number of grains per spike (25) and difference between them was significant (figure 3).

1000 grain weight : The results showed that, the effect of chemical fertilizer on 1000 grain weight was significant (table 1).The comparison means values for 1000 grain weight showed that application of Urea had a highest (39 g) and the control treatment had the lowest 1000 grain weight (32 g) (figure 4).

Grain yield :The results showed that, the effect of chemical and biological fertilizers on grain yield were significant (table 1). The comparison means values for chemical fertilizer showed that application of Urea had a highest (560 $g.m^{-2}$) and the control treatment had the lowest grain yield (500$g.m^{-2}$) (figure 5). Also, among the biological fertilizers application of Nitroplus has the highest (540$g.m^{-2}$) and control treatment had the lowest (490$g.m^{-2}$) grain yield and differences between them were significant (figure 6).

Biomass yield :The effect of chemical and biological fertilizers and interaction between them on biomass yield were significant (table 1). The comparison of the mean values of the biomass yield for interaction between chemical and biological fertilizers showed that application of Urea and Amunium with Nitroplus and Nitrokara had the highest (910$g.m^{-2}$) and the control treatment had the lowest (760$g.m^{-2}$) biomass yield (figure7).

Harvest index (HI): Results showed that, the effect of biological fertilizers on harvest index was significant only (table 1). The comparison of the mean values of HI showed that between all treatment of biological fertilizers, non-application of any of biofertilizers treatment(control) had the highest harvest index(figure 8).

The results of this study showed that yield and yield components of wheat affected by chemical and biological fertilizers significantly (table1).The positive effect of biofertilizer on yield and yield components of many crops were reviled by many authors (3, 4 and 7). Application of chemical and biological fertilizers increased the average number of spikelet per spike, number of grin per spike, 1000 grain weight, biomass and grain yield and HI rather than non-application of them. This may resulted from its ability to increase the availability of nutrients especially the specialty of the calcareous nature of the soil which cause decreasing on the nutrients availability, results agree with (12 and 19).In the present study, significant differences were observed among chemical and biological fertilizers regarding the average yield and yield components of wheat. (20) conducted experiments to investigate the effect of biofertilizers on the growth and yield of wheat and indicated that biofertilizer inoculation and produced significant increment in all growth characters. In the other research Bahraniet al., (5) carried out studies on the effect of biofertilizer application on wheat productivity and reported that there have been positive effects of inoculating wheat seed with various biofertilizer sources on the crop yields. Also, Mahdi et al., (14) ascertained the effect of biofertilizers on the growth and seed yield of wheat and the results showed that dual inoculation of wheat seeds with biofertilizers (Azotobacter and yeast) was superior to single ones. High grain yield in common wheat achieved in combined application of chemical fertilizer in this study. Both application of chemical and biofertilzer increase main yield components such as biomass yield. However, The comparison of the mean values of the biomass yield for interaction between chemical and biological fertilizers showed that application of Urea and Amunium with Nitroplus and Nitrokara had the highest and the control treatment had the lowest biomass yield (figure7). (1) reported that under organic and biofertilization, the highest dry matter accumulation in shoot system and spikes and the highest yield and yield components recorded in Gemmiza10 cultivar fertilized with 20 m3/fad and inoculated with yeast and Azotobacter. Beyranvand et al (7) reviled that application nitrogen and phosphate biofertilizers increased yield and yield components of maize under Boroujerd environmental condition. They suggested that effect of nitrogen and phosphate biofertilizers were evaluated positively, there were an increase in plant height, ear weightand number of grain per ear, grain yield and biomass yield. Sharifi and Haghnia (18) showed that the using of biofertilizers increased grain yield of wheat .Azimi et al (4) found that that application nitrogen and phosphate biofertilizers increased yield and yield components of barley under Boroujerd environmental condition.Sharaf (17) showed that application of mixtureAzotobacterwithAzospirilluminorganic N-fertilizer, in combination withusing ofmycorrhiza (VAM),improved growth and yield of both datura andammi plants.

CONCLUSION

In final our results showed that for biological treatment maximum number of spikelet per spike was recorded at application of Nitroxinbiofertilizer. Interaction effect of chemical and biological fertilizers shoed that application of Urea and Amunium with Nitroplus and Nitrokara had the highest biomass yield. Also, control treatment had the highest harvest index. In final our results showed that application of chemical and biological fertilizers increased yield and yield components of wheat but chemical fertilizers specially Urea fertilizer increased them more than biological fertilizers.

REFERENCES

1. Ahmed, M. A., Ahmed, A. G. Mohamed, M. H. and Tawfik, M. M. (2011). Integrated Effect of Organic AndBiofertilizers On Wheat Productivity In New Reclaimed Sandy Soil. Research Journal of Agriculture and Biological Sciences, 7(1): 105-114
2. Akaram.S.S.Muhammed , H.UR.R. MEMON2, S.K. Baloch, Khalid Abdalla Osman and Suliman A. Ibrahim Ali. (2013). Effect of Bio-organic And Inorganic Fertilizers On The Growth And Yield Of Wheat (Triticumaestivum L.). Persian Gulf Crop Protection. 2(4):15-24.
3. Azimi SM, Farnia A, Shaban M, Lak M. (2013a). Effect of different biofertilizers on Seed yield of barley (Hurdeom vulgar L.), Bahman cultivar. International journal of Advanced Biological and Biomedical Research. Volume 1, Issue 5: 538-546.
4. Azimi SM, Nabati E, Shaban M Lak M. (2013b). Effect of N and P bio fertilizers on yield components of barley. International journal of Advanced Biological and Biomedical Research. Volume 2, Issue 2: 365-370.
5. Bahrani, A. Pourreza, J. and HaghJoo, M. 2010. Response of Winter Wheat to Co-Inoculation with Azotobacter and ArbescularMycorrhizal Fungi (AMF) under Different Sources of Nitrogen Fertilizer. American Eurasian Journal. Agriculture and Environmental Science, 8 (1): 95-103.
6. Belimov AA, Kojemiakov PA, Chuvarliyeva CV. 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphatesolubilizing bacteria. Plant and Soil, 17: 29–37.
7. Beyranvand H, Farnia A, Nakhjavan SH, Shaban M. 2013. Response of yield and yield components of maize (Zeamaiz L.) to different bio fertilizers. International journal of Advanced Biological and Biomedical Research. 1 9; 1068-1077.
8. El-Ghadban EAE, Shalan MN, Abdel-Latif ATA. 2006. Influence of biofertilizers on growth, volatile oil yield and constituents of fennel (FoeniculumvulgareMill.). Egyptian Journal of Agricultural Research, 84: 977–992.
9. Goel, A.K., R.D.S. Laura, G. Pathak, G. Anuradha and A. Goel, 1999 Use of bio-fertilizers: potential, constraints and future strategies review. Int. J. Trop. Agric., 17: 1–18.
10. Javaid, A., Tehmina, A. and Bajwa, R. 2002. EM and VAM technology in Pakistan XII: growth, nodulation and VA mycorrhizal response of Phaseolus vulgaris L. to long-term EM application. Pakistan Journal of Phytopathology, 14 (1): 57-61.
11. Karmakar, S., Lague, C. Agnew, J. and Landry, H. 2007. Integrated decision support system (DSS) for manure management. A review and perspective Computers and Electronics in Agriculture, 57: 190- 201
12. Kucey, R.M.N., H.H. Janzen M.E. Leggett. 1989. Microbially mediated increases in plantavailable phosphorus. Ad. Agron., 42: 199–228.
13. Lin W, Okon Y, Hardy RWF. 1983. Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with Azospirillumbrasilense. Appl. Environ. Microbiol. 45:1775-1779.
14. Mahdi, S. S., Hassan, G. I. S. Samoon, A. Rather,H. A. Dar, S.A. and Zehra, B. 2010. Bio-Fertilizers in Organic Agriculture. Journal of Phytology, 2(10): 42-54.
15. SAS Institute 2002. JMP statistics and graphics guide. SAS Institute Inc., Cary, NC.
16. Savin R, Slafer GA. 1991. Shading effects on the yield of an Argentinian wheat cultivar. J. Agric. Sci. 116, 1–7.

17. Sharaf, M.S., 1995. Response of some medicinal plants to inoculation with symbiotic N2-fixers. Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Egypt.
18. Sharifi, Z. and G.H. Haghnia, 2006. Effect of NitroxinBiofertilizer Application on Grain Yield and Yield Components of Wheat (cvSabalan). 2nd National Conference on Agro Ecology in Iran, Gorgan, Iran. Pp. 1-3.
19. Tiwari VN, Lehri LK, Pathak AN. 1989. Effect of inoculating crops with phosphomicrobes. Experimental Agriculture, 25: 47–50.
20. Zaki, M., Nabila, M. Hassanein, S. and Gamal El-Din, K.M. 2007. Growth and yield of some wheat cultivars irrigated with saline water in newly cultivated land as affected by biofertilization. Journal. of Applied Sciences, 1121-1126

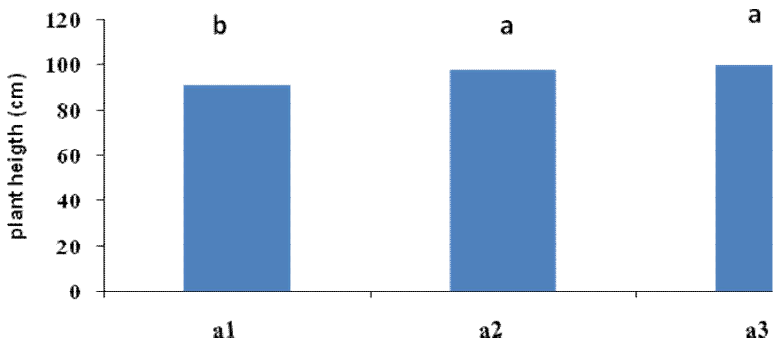

Figure 1. Mean comparison effect of chemical fertilizers on plant height in wheat.

(a1= control, a2= Ammonium nitrate, a3=Urea)

Means by the uncommon letter in each column are significantly different (p<0.05).

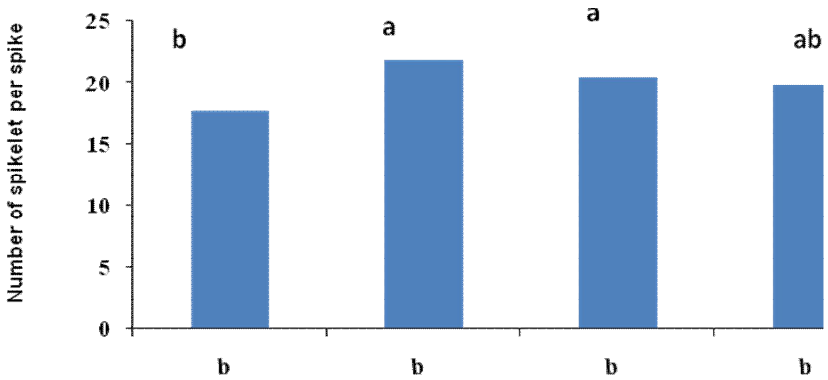

Figure 2. Mean comparison effect of biological fertilizers on number of spikelet per spike in common wheat.

(b1=control, b2=Nitroxin, b3=Nitroplus and b4= Nitrokara)

Means by the uncommon letter in each column are significantly different (p<0.05).

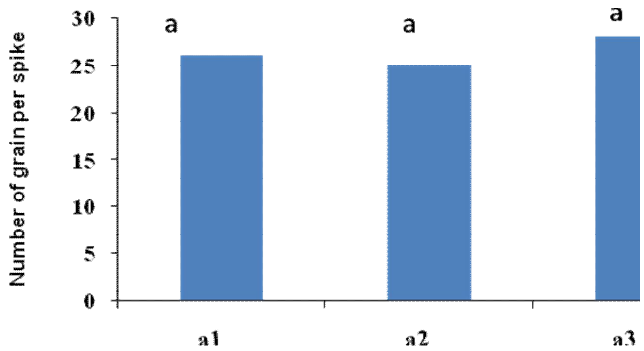

Figure 3. Mean comparison effect of chemical fertilizers on number of grain per spike in wheat.
(a1= control, a2= Ammonium nitrate, a3=Urea)
Means by the uncommon letter in each column are significantly different (p<0.05).

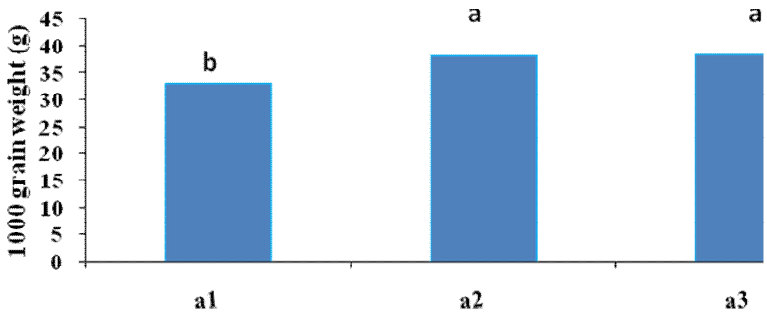

Figure 4. Mean comparison effect of chemical fertilizers on 1000grain weight in wheat.
(a1= control, a2= Ammonium nitrate, a3=Urea)
Means by the uncommon letter in each column are significantly different (p<0.05).

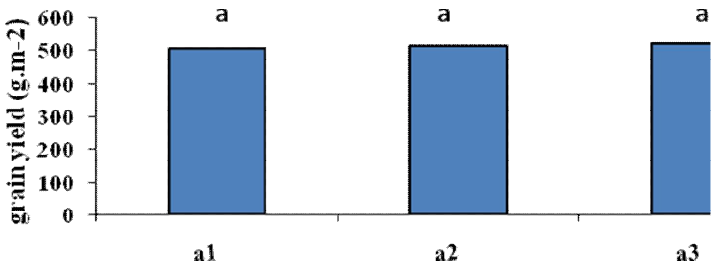

Figure 5. Mean comparison effect of chemical fertilizers on grain yield in wheat.
(a1= control, a2= Ammonium nitrate, a3=Urea)
Means by the uncommon letter in each column are significantly different (p<0.05).

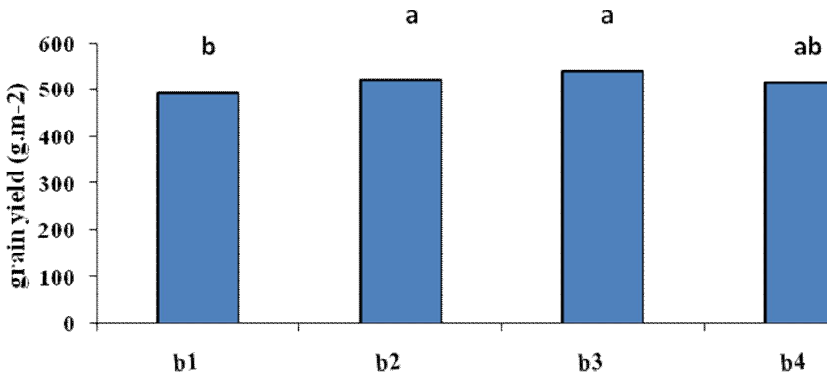

Figure 6. Mean comparison effect of biological fertilizers on grain yield in wheat.
(b1=control, b2=Nitroxin, b3=Nitroplus and b4= Nitrokara)
Means by the uncommon letter in each column are significantly different (p<0.05).

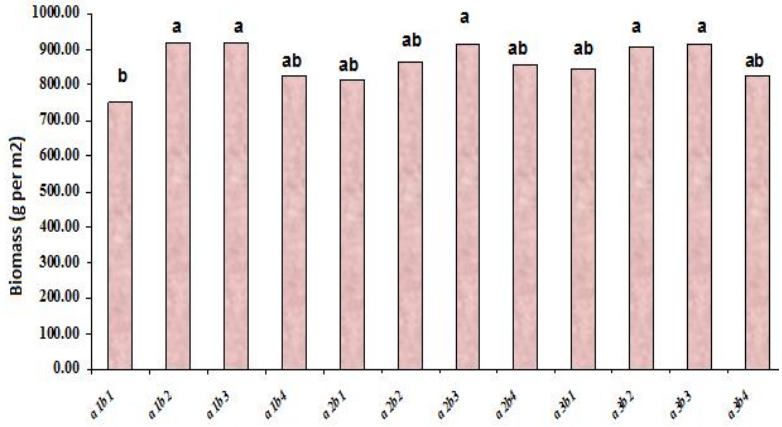

Figure 7. Mean comparison for interaction effect of chemical and biological fertilizers on Biomass in wheat.
(a1= control, a2= Ammonium nitrate, a3=Urea, b1=control, b2=Nitroxin, b3=Nitroplus and b4= Nitrokara)
Means by the uncommon letter in each column are significantly different (p<0.05)

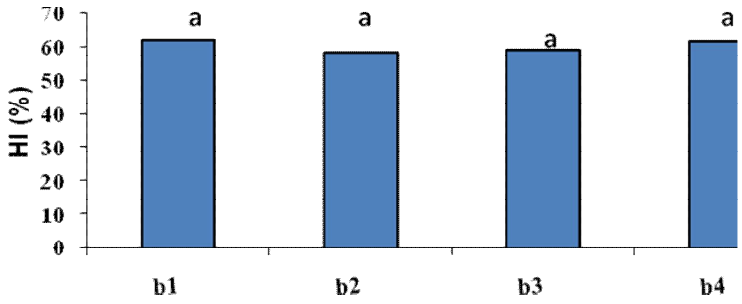

Figure 8. Mean comparison effect of biological fertilizers on harvest index in wheat.
(b1=control, b2=Nitroxin, b3=Nitroplus and b4= Nitrokara)
Means by the uncommon letter in each column are significantly different (p<0.05).

Table 1. Analysis of variance (mean squares) for yield and yield components of wheat under application chemical and biological fertilizers

S.O.V	df	Plant height	Number of spikelet per spike	Number of grain per spike	1000 grain weight	Grain yield	Biomass yield	HI
R	2	67	5.8	16.44	6800	1102	43000	991
Chemical fertilizer(A)	2	273*	14	107*	1326	10232**	26000	696
Biological fertilizer(B)	3	176	26*	28	24703**	74988**	32000	3584*
A*B	6	30.5	14.5	14	2125	15214	240000*	259
Error	18	124	6.7	29	4989	4456	56000	1112
CV	-	10.1	15.7	14.9	9.04	12.27	10.94	6.64

ns: Non-significant, * and **:Significant at 5 and 1% probability levels, respectively.

Indian Journal Of Natural Sciences *www.tnsroindia.org. © IJONS*

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Humic Acid and Nitrogen Fertilizer on Yield and Yield Components of Tomato (*Lycopersicon esculentum* L.)

Amin Farnia[1]* and Mohammad Reza Rezaei[2]

[1]Assistant Professor of Department of Agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.
[2]M.Sc student of Department of Agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.

Received: 25 Mar 2015 Revised: 27 Apr 2015 Accepted: 30 May 2015

***Address for correspondence**
Amin Farnia
Assistant Professor of Department of Agriculture,
Boroujerd branch,
Islamic Azad University, Boroujerd, Iran
E-mail: Aminfarnia@yahoo.com.

ABSTRACT

A filed experiment was laid out in order to study on effect of humic acid and nitrogen fertilizer on yield and yield components of tomato (LycopersiconesculentumL.). This study was performed in the Faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch, Boroujerd, Iran (Kermanshah region) during the growing seasons 2013- 2014. The experiment was laid out in a factorial based on randomized block design with three replications. Treatments were 35% humic acid in five levels such as (0, 100, 200, 300 and 400 kg.ha-1) and pure Azot in five levels such as (0, 50, 100, 150 and 200 kg.ha-1). Results showed that the application of humic acid and nitrogen fertilizer were significant of all traits of tomato but effect of humic acid on fruit length was not significant. Application of 400 kg per ha humic acid had the highest plant height and stem diameter. Also results showed that application of 300 kg per ha humic acid had the highest number of fruit per plant, fruit weight, fruit diameter and fruit yield. However application of nitrogen fertilizer increased yield and yield components of tomato. In final results of the present study reviled that application of humic acid and nitrogen fertilizer increased yield and yield components of tomato.

Key words: Humic acid, nitrogen fertilizer and tomato.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most important fruit vegetables, which due to high nutrient value, is in the second rank in viewpoint of level under cultivation and consumption (6). Tomato is one of the most important agricultural plants in semi-arid and the Mediterranean areas. Tomato cultivation is very common as a major and productive crop in many parts of Iran (15). It is rich in vitamins A, C (Block, 1992), B1, B2 and B3. Antioxidant and anticancer effects of tomato reflect the importance of its consumption (19). Lycopene in addition to neutralizing ability to singlet oxygen (19) and antioxidant properties than beta-carotene (beta-carotene twice) and alpha-tocopherol (alpha-tocopherol twice), is able to prevent heart disease, cardiovascular disease and various cancers especially prostate, lung and stomach.

The using of nitrogen fertilizer was increased the rate of photosynthesis in the leaves, thus increasing of the rate, the more seeds was created (9). Ammonia with 82% nitrogen has the highest nitrogen amount between nitrogen fertilizers and in many countries is directly injected to soil as the fertilizer however in the central parts of Iran less is used because of soil moisture deficit as well as possibilities deficiency (8).Nitrogen is one of the most principle necessary nutrient elements for plants and other living beings so that after water, the plant need to this factor more than other effective factors for plant growth. Despite nitrogen has been allocated about 79% voluminal of atmosphere but many plants involved to nitrogen deficiency due to deficit of organic matter in these soils, specially the plants that grow in dry and semi-dry regions (17).

The using of humic acid using in the soil increase the absorption of nutrients from the soil and plant nutrient efficiency (1).Humic acids can directly cause the positive effects on plant growth. Shoot and root growth is stimulated by humic acid, but its effect is more prominent on the roots. Humic acid increased root content and caused the root system effectiveness. Also the humic acid increases the absorption of nitrogen, potassium, calcium, magnesium and phosphorus by plants (Sabzevari et al., 2009). Humic substances are generated through organic matter decomposition and employed as soil fertilizers in order to improve soil structure and soil microorganisms. The use of humic substances (HS) to improve crop growth has been the subject of a substantial body of research over decades. HS refers to a complex, heterogeneous mixture of organic materials arising from the decay of plant and animal residues (14).

Foliar sprays of these substances also promote growth, and increases yield and quality in a number of plant species (12) at least partially through increasing nutrient uptake, serving as a source of mineral plant nutrients and regulator of their release (4).Humic acid effectively improves soil fertility and crop production especially in poor soils and alkaline-calcareous soils (17). Some researchers believe that the effect of organic matter in soils with low organic matter as a result of production of humic acid and folic acid and improvement of chelating the elements. HakimiMeybodi et al.(10) reported the use of humic acid in lawn cv. ‘Speedy Green’ increased uptake of potassium, zinc and iron, however, significantly different from control at potassium uptake were not observed.The researcher by investigating the effect of different levels of humic acid on wheat concluded that humic acid levels had significant differences between stem weight and plant height and the amount of nitrogen in wheat growth (20). In a study the effect of humic acid on some grass was studied and was found that using of humic acid increased the rangeland herb. Therefore, the main aim of present study was evaluation the effects of nitrogen and humic acid on yield and yield components of tomato.

MATERIAL AND METHODS

A field experiment was laid out to study on evaluation the effects of nitrogen and humic acid on yield and yield components of tomato. This study was performed in the Faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch, Boroujerd, Iran (Kermanshah region) during the growing seasons 2013- 2014. The

experiment was laid out in a factorial based on randomized block design with three replications. Treatments were 35% humic acid in five levels such as (0, 100, 200, 300 and 400 kg.ha^{-1}) and pure Azot in five levels such as (0, 50, 100, 150 and 200 kg.ha^{-1}). Plant to plant distance was 25 cm and during growing stages weeds were control. After harvesting yield and it components were determined.

The statistical analyses to determine the individual and interactive effects of treatments were conducted using JMP 5.0.1.2 (18). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

RESULTS AND DISCUSSION

Plant height : Analysis of variance of results showed that, the effect of humic acid and nitrogen fertilizer on plant height was significant (table 1). The comparison of the mean values showed that application of 400 kg per ha humic acid had the highest (92cm) and the control treatment had the lowest (77cm) plant height and difference between them was significant (figure 1). Between nitrogen fertilizer treatments application of 200 kg per ha treatment had the highest (100cm) plant height.

Stem diameter : Results results showed that, the effect of humic acid and nitrogen fertilizer on stem diameter was significant (table 1). The comparison of the mean values showed that application of 400 kg per ha humic acid had the highest (4cm) and the control treatment had the lowest (3cm) stem diameter and difference between them was significant (figure 2). Between nitrogen fertilizer treatments application of 100 kg per ha treatment had the highest (3.5cm) stem diameter.

Number of fruit per plant : Results results showed that, the effect of humic acid and nitrogen fertilizer on number of fruit per plant was significant (table 1). The comparison of the mean values showed that application of 300 kg per ha humic acid had the highest (38) and the control treatment had the lowest (23) number of fruit per plant and difference between them was significant (figure 3). Between nitrogen fertilizer treatments application of 150 kg per ha treatment had the highest (29) number of fruit per plant.

Fruit weight : The effect of humic acid and nitrogen fertilizer on fruit weight was significant (table 1). The comparison of the mean values showed that application of 300 kg per ha humic acid had the highest (75g) and the control treatment had the lowest (62g) fruit weight and difference between them was significant (figure 4). Between nitrogen fertilizer treatments application of 200 kg per ha treatment had the highest (79g) fruit weight.

Fruit length : The effect of nitrogen fertilizer on fruit length was significant (table 1). Between nitrogen fertilizer treatments application of 100 kg per ha treatment had the highest (6.5cm) fruit length.

Fruit diameter : The effect of humic acid and nitrogen fertilizer on fruit diameter was significant (table 1). The comparison of the mean values showed that application of 300 kg per ha humic acid had the highest (5.7cm) and the control treatment had the lowest (5cm) fruit diameter and difference between them was significant (figure 5). Between nitrogen fertilizer treatments application of 150 kg per ha treatment had the highest (5.8cm) fruit diameter.

Fruit yield : The effect of humic acid and nitrogen fertilizer on fruit yield was significant (table 1). The comparison of the mean values showed that application of 300 kg per ha humic acid had the highest (90 ton per ha) and the control treatment had the lowest (46 ton per ha) fruit yield and difference between them was significant (figure 6). Between nitrogen fertilizer treatments application of 150 kg per ha treatment had the highest (88 ton per ha) fruit yield.

Results of present study showed that the application of humic acid and nitrogen fertilizer were significant of all traits of tomato but effect of humic acid on fruit length was not significant (table 1). The humic acid increases the absorption of nitrogen, potassium, calcium, magnesium and phosphorus by plants (9 and 16). Also humic acids can directly cause the positive effects on plant growth. Shoot and root growth is stimulated by humic acid, but its effect is more prominent on the roots. Humic acid increased root content and caused the root system effectiveness. Plant height was affected by humicacid and nitrogen fertilizer. In the present study application of 400 kg per ha humic acid had the highest and the control treatment had the lowest plant height and difference between them was significant.Humic substances are generated through organic matter decomposition and employed as soil fertilizers in order to improve soil structure and soil microorganisms (11).

Application of 300 kg per ha humic acid had the highest (5.7cm) and the control treatment had the lowest (5cm) fruit diameter and difference between them was significant (figure 5). However, Yildirim (21) have reported a significant enhancement in fruit diameter and length as a result of exogenous HA application in tomato but the author did not find any significant difference between soil HA application and control in terms of fruit diameter.The application of 300 kg per ha humic acid had the highest and the control treatment had the lowest fruit weight and difference between them was significan. Between nitrogen fertilizer treatments application of 200 kg per ha treatment had the highest fruit weight. To elucidate the effects of humic substances and bio-stimulators, several hypotheses suggesting the formation of a complex between these substances and mineral ions, their involvement in the enhancement of enzyme catalysis, their influence of stimulating respiration, photosynthesis and nucleic acid metabolism, and their hormonal activity have been reported (7).

Application of humic acid and nitrogen fertilizer increased fruit weight and fruit yield of tomato. Application of 300 kg per ha humic acid had the highest (90 ton per ha) and the control treatment had the lowest fruit yield and difference between them was significant. Application of 150 kg per ha nitrogen fertilizer had the highest fruit yield. Collectively, the reported findings that HA treatments improved growth and some fruit characteristics of various plants including cucumber, tomato, eggplant and pepper (2, 13 and 21) were confirmed in our study.

It can be concluded from the results of this study that application of humic acid and nitrogen fertilizer can be safely used within the applied concentrations with a positive effect on plant growth, fruit set and improvement of production of tomato plants. In conclusion, application of HA and nitrogen fertilizer can result in an increase and improvement in the tomato fruit yield. The results could be due to the reported enhancement in the growth of cucumber in response to the incorporation of HA into plant growth media (3 and 5). Collectively, the results of the present study suggest that HA and nitrogen treatment might efficiently be utilized to obtain higher fruit yield and it components in tomato.

REFERENCES

1. Adani, F., Genevini, P., Zaccheo, P., Zocchi, G. 1998. The effect of humic acid on tomato plant growth and mineral nutrition. Journal of Plant Nutrition. 21: 561–575.
2. Arancon NQ, Edwards CA, Lee S, Byrne R (2006). Effects of humic acids from vermicomposts on plant growth. European J. Soil Biol., 42: 65-69.
3. Atiyeh RM, Edwards CA, Metzger JD, Lee S, Arancon NQ (2002). The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Biores. Technol., 84: 7-14.
4. Atiyeh RM, Edwards CA, Metzger JD, Lee S, Arancon NQ (2002). The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Biores. Technol., 84: 7-14.
5. Block G B (1992). Fruit vegetables and cancer prevention: a review of the epidemiological evidence Journal Nutrition. Cancer.18: 1-29.
6. Daneshvar MH. 2000. Vegetables growing. Shahid-Chamran University Press.

7. Dell'Agnola, G. and S. Nardi, 1987. Hormone-like effect and enhanced nitrate uptake induced by depolycondensedhumic fractions obtained from Allolobophorarosea and A. caliginosafaeces. Biology and Fertility of Soils, 4: 115-118.
8. Ejraei AK. 2009. Soil fertility. Text booklet of Islamic Azad University of Jahrom, Khayam Press.
9. Ghosh, D.K., Roy and Malic, S.C. 1t981. Effect of fertilizers and spacing on yield and other characters of black cumin (Nigella sativa L.). Indian Agric. 25: 191-197.
10. Hakimimeibodi, N., M. Kafi, A. Nikbakht and F. Rajali. 2011. Effect of humic acid on qualitative and quantitative characteristics of Speedy green grass. Journal of Horticultural Science. 42 (4): 403-412.
11. Halime, O.U., U. Husnu, K. Yasar and P. Huseyin, (2011). Changes in fruit yield and quality in response to foliar and soil humic acid application in cucumber. Scientific Research and Essays, 6(13): 2800-2803.
12. Karakurt Y, Unlu H, Unlu H, Padem H (2009). The influence of foliar and soil fertilization of humic acid on yield and quality of pepper. ActaAgriculturaeScandinavica Section B Plant Soil Science, 59 (3): 233- 237.
13. Karakurt Y, Unlu H, Unlu H, Padem H (2009). The influence of foliar and soil fertilization of humic acid on yield and quality of pepper. ActaAgriculturaeScandinavica Section B Plant Soil Science, 59 (3): 233- 237.
14. MacCarthy, P., C.E. Clapp, R.L. Malcolm, and P.R. Bloom. 1990. An introduction to soil humicsubstances, p. 1–12. In:
15. MacCarthy, P., C.E. Clapp, R.L. Malcolm, and P.R. Bloom (eds.). Humic substances in soil and crop sciences: Selected readings. Amer. Soc. Agronomy, Madison, WI.
16. Mokhtari I, Abrishamchi P, Ganjeali A (2008). The effects of Calcium on amelioration of injuries salt stress on seed germination of tomato (Lycopersiconesculentom L.). Iranian Journal of Sciences and Food Technology. 22(1): 89-100.
17. Rajaei M. 2010. Plant nutrition. Text booklet of Islamic Azad University of Jahrom, Khayam Press.
18. SAS Institute 2002. JMP statistics and graphics guide. SAS Institute Inc., Cary, NC.
19. Soni J (2003). Separation of carotenoids from fruits and vegetables, US2003/0180435.
20. Tahir, M.M., Khurshid, M., Khan, M.Z., Abbasi, M.K., Kazmi, M.H. 2011. lignite- derived humic acid effect on growth of wheat plants in different soils, Pedosphere. 21: 124-131.
21. Yildirim E (2007). Foliar and soil fertilization of humic acid affect productivity and quality of tomato. ActaAgriculturaeScandinavica Section B-Soil Plant Science, 57: 182-186.

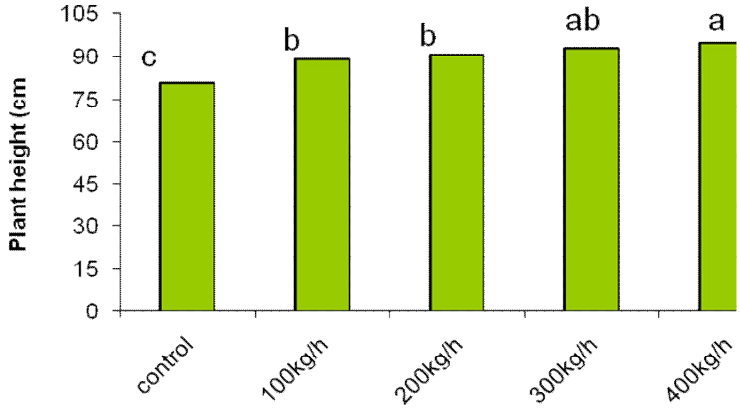

Figure1. Effect of humic acid application on plant height

Means by the uncommon letter in each column are significantly different (p<0.05).

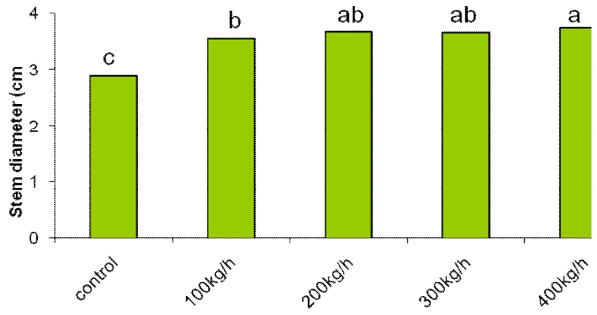

Figure2. Effect of humic acid application on stem diameter
Means by the uncommon letter in each column are significantly different (p<0.05).

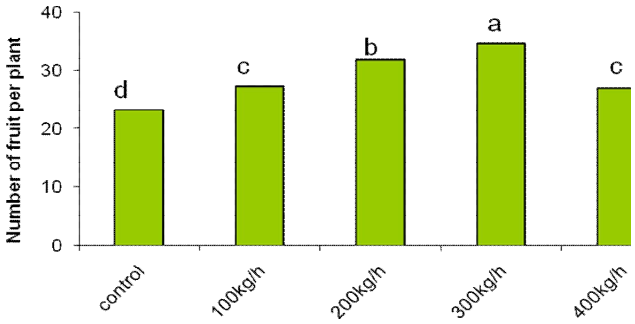

Figure3. Effect of humic acid application on number of fruit per plant
Means by the uncommon letter in each column are significantly different (p<0.05).

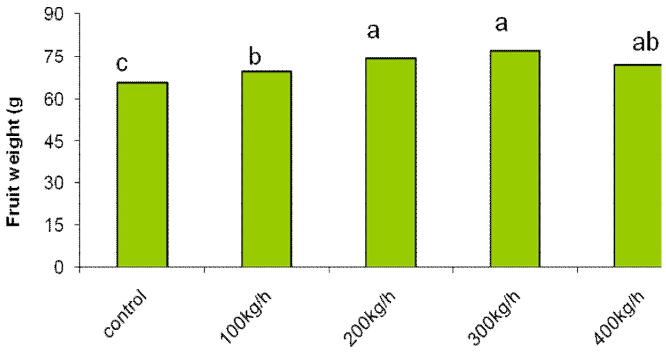

Figure4. Effect of humic acid application on fruit weight
Means by the uncommon letter in each column are significantly different (p<0.05).

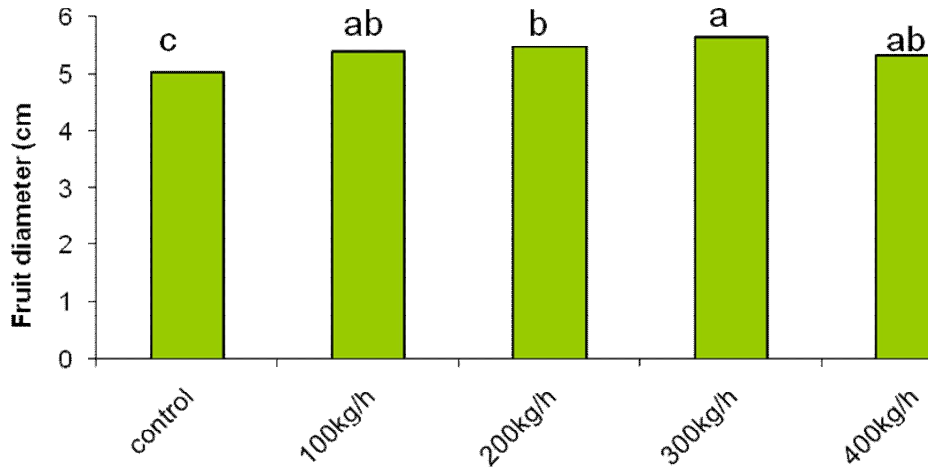

Figure5. Effect of humic acid application on fruit diameter
Means by the uncommon letter in each column are significantly different (p<0.05).

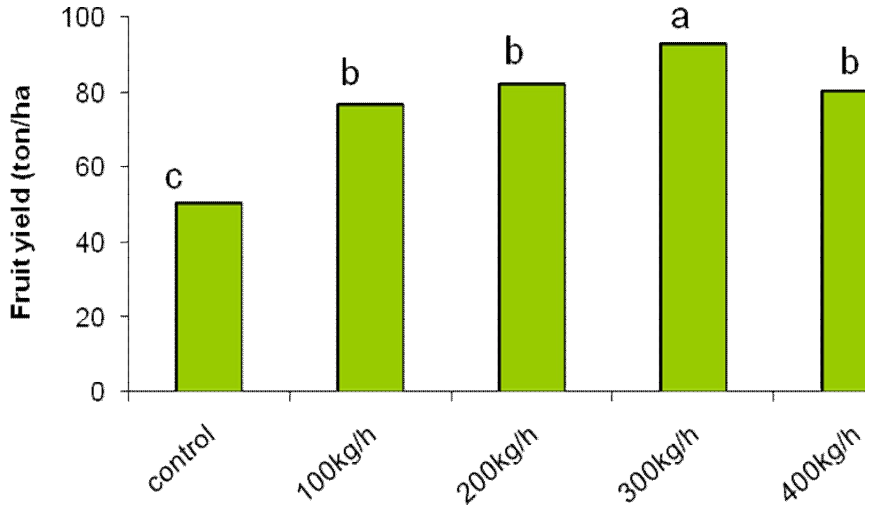

Figure6. Effect of humic acid application on fruit yield
Means by the uncommon letter in each column are significantly different (p<0.05).

Table 1. Analysis of variance (mean squares) for yield and yield components of tomato under application of humic acid and nitrogen fertilizer

S.O.V	df	Plant height	Stem diameter	number of fruit per plant	fruit weight	fruit length	fruit diameter	fruit yield
R	2	106	0.15	178	54	2	1.7	1042
Humic acid (H)	4	440**	0.71**	304**	118**	0.49	0.78*	3300**
N fertilizer(N)	4	72*	0.13*	593**	82**	1.5*	3.3**	1519**
H*N	16	31	0.02	43	31	0.27	0.23	340
Error	48	10	0.02	9.7	12	0.19	0.23	106
CV(%)		3.6	4.5	10	4.8	7.3	9	13

* and **:Significant at 5 and 1% probability levels, respectively.

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Effective use from Urban and Industrial Waste Water in Agriculture

Mehrzad Mostashari Mohasses

Scientific Member of Agricultural and Natural Resources Education and Research Center of Qazvin 3415677566-Iran.

Received: 28 Mar 2015 Revised: 25 Apr 2015 Accepted: 30 May 2015

***Address for correspondence**
Mehrzad Mostashari Mohasses (PhD)
Scientific member of Agricultural,
Natural Resources Education,
Research Center of Qazvin, 3415677566-Iran.
Mobile: 09121815587,
E-mail: mm_mohasses@yahoo.com

ABSTRACT

In order to effective use of urbanand industrial wastewater in agriculture,a plan of lands in Alborz industrial city on 3 products (wheat, barley and maize) was carried out. First, were prepared Composite sample of soil from every 10 hectares, 0-15 and 15-30 cm of depth with 2 reps. after cultivation, Desired products were irrigated with wastewater sources. Analysis of soil showed that in lands were irrigated with waste water in Alborz industrial city, the amount of heavy metals, especially cadmium, zinc,Pb, Cu and also P increased compared to control lands (without irrigation with waste water). During much use of the waste water, the amount of these elements will be higher than world standard. After sampling the plant was found that in the analysis of seed plants studied, the amount of heavy metal reported negligible and this shows that movement elements of root to shoot and finally seed plant is slow. On the other hand, the use of waste water leads to increasing the amount of soil salinity. Therefore in order to correct use of waste water and environmental protection and development of water resources for irrigation agriculture and the use of urban and industrial wastewater, Filtration and removal of salts and elements Harmful substances seem necessary.

Key words: industrial wastewater, cadmium, zinc,Pb, Cu, environmental protection.

INTRODUCTION

Unusual water volume includingurban and industrial wastewater in Iran (statistics 1996) is 3/36 billion cubic meters per year (2/5 billion cubic meters of Urban sewage wastewater). The amount of wastewater in 2001 has reached 4/5 billion cubic meters per year and it is expected that wastewater volume in 2011 reach to 7 billion cubic meters per year. Therefore it is necessary that status this wastewater in the form of long-term researches in the world including Iran seriously be considered.Alborz industrial city area is 900 hectares and it has 400 great Industrial units and the amount of waste water that they produce, is 40-35 million cubic meters per day. The area of agricultural lands irrigated with waste water the industrial city is about 200 hectares and according to reporting charge company Alborz industrial wastewater, the amount of waste water indicators after filtration as follows:

Acidity or pH is equal 6- 7/8, TDS is equal 2000-2400 mg/Lit, TSS is equal 450-850. Other factors are the amount of oxygen required for biochemical reactions (BOD) is equal to 450 to 700 and the oxygen required for chemical reactions (COD) is equal to 800-1800 that after filtration, the amount of BOD will decrease to 100; the amount of COD will decrease to 200. On the other hand, Increase the growth of World and the need to greater production and also limitations of Water Resources

And indiscriminate use of them, especially in arid and semi-arid and also huge volumes of wastewater produced in cities and the need for their appropriate excretion has increased importance of the use of waste water in agriculture and artificial recharge of groundwater aquifers (Asona, Cotruvo.2004; Haruvy, 1997).

Increasing toxic elements in the soil when entering pollutants is one of the most important environmental issues (Chang, 1984). For decades, Waste water and sewage sludge in agricultural land are used as a supplemental source of irrigation water, fertilizers and soil modifiers (Page, 1995).

Using them in the short term may not cause toxicity in the plant (Page, 1974), But long-term use of these wastewater or in other words uncontrolled entry of heavy metals in soils may increase these elements in the soil and Plants cultivated in these soils absorbs these elements and easily enters to food chain. Miterly about the release of wastewater to agricultural land says that although Sewage disposal in land has lowest price but its adverse effects can be accumulation of metals such as chromium, nickel and cadmium that first this elements will pollute soil (Torabian and Baghori, 1996).

According to the report (Ross, 1995), concentration of heavy metals in soils is accordance with Table 1.

Studies Sidle (1977) showed that Cadmium added to the soil due to the use of waste water often does not exist in the soil profile. Also in most studies, have seen Pb accumulation in surface layers of soil The main cause of accumulation of elements (Zn, Pb and Cd) in the soil surface layer is absorption high capacity these third element by soils (Carey, 1987).

This feature is the result of the reaction of these elements with the components of the soil solid phase including silicate clay, Oxides and hydroxides of metals particularly iron and aluminum, Amorphous minerals of lime and organic matter and the formation of strong links with these components.

Aphioni et al., (1998) reported that addition of sewage sludge to soil significantly increases the concentrations of extractable Cu, Zn and Pb by EDTA in the soil and absorption these metals in plant. Khaiambashi (1997) in a study showed that Application of sewage sludge leads to an increase in the total amount and absorbable Zn, Cu, Mn, Pb and Ni in the soil.

In evaluation the effects Urbansewage wastewater water feeding in forage maize cultivation was founded that In surface layer of agricultural land, % organic matter, absorption P, absorption K and heavy metal including Cu and Zn increased. The use of urbanwastewater in olericulture land in Hamedan city showed that concentrations of heavy metals in vegetables (except Cu and Zn) were less than the permissible limit.

Research results (Rahmani, 2005) showed that in all wastewater irrigated soil sample, %organic matter, absorption P, absorbable concentration in all heavy metals tested was beyond from values of these parameters in soils irrigated by wells water. (Elliot & Stevenson, 1986) In many reports showed that wastewater have the ability to increase the concentration of heavy metals in the soil and in some cases reach to harmful border?

Hosseinpur et al., 2005 a study on Wastewater disposal showed that over time the test has a significant effect on the increase the amount of TOC, EC, cations, anions, Ni and decrease in pH. JafarzadehHaghighi (2005) during effect evaluation of using from wastewater Shiraz city in irrigation of crops on increasing the concentration of heavy metal in soil and some products believes that discharge wastewater into seasonal river Shiraz lead to increase The concentration of heavy metals above the exposure limits for use in irrigation of crops and the average Pb, iron, Cu and Cd in studied products is more than recommended amounts and it is understood that Direct application of sewage discharged into the seasonal river Shiraz Due to various metal elements in the long term can lead to Increasing contamination of soils adjacent to the river and the transfer the some pollutants elements in agriculture.

MATERIALS AND METHODS

First, Location and land surface and the type of productswhich are irrigated with the waste water, were identified. Then, three plants that are major crops and its land irrigate with the wastewater, were prepared at the level of 100 to 200 hectares based on uniformity fields and maximum from every ten hectares were prepared two soil samples from the bottom of 0-15 and 15-30 cm to two replications as mixture. (It should be noted that in Qazvin,adjacent lands ofAlborz Industrial city are about 200 hectares and they are irrigated with the wastewater of this Industrial city and Major crops are corn, wheat, barley, and as scatter of sugar beet which was selected for the above research project).

Then, in order to evaluate the effect of the use of waste water on crops from ten hectares of land area that are planted to similar plants and irrigate by Non-waste water sources, were taken samples as control plots. Of each segment of ten hectares irrigated with the waste waterand also control plots, according to the type of product, uniformity and safety of the plant before crop harvest were collected A sample the plant seed is composed In two Repeat.

Thus from every ten hectares plot of original treatments were obtained four soil samples and two plant samples. Analysis of Desired for soils include EC, pH, phosphorus, organic matter, calcium carbonate, soil texture and heavy metals (Cu, Pb, Ni, Zn and Cd) and for plant samples include phosphorus and heavy elements. Measurement method the absorption amounts of heavy metals in soil were carried out using DTPA and determining its amount in plant to method of wet ash in mixed three acids.

Finally, the results of analysis of samples of soil and plants in each region were examined and were analyzed.

RESULTS

The concentration of heavy metals Pb, Cu, Cd and Zn and phosphorus in soils irrigated with wastewater is several times the amount of these elements in soils of control fields. Also, the amount of soil salinity in fields irrigated with wastewater is About 2 times of the salinity soil in control fields. The following levels of heavy metals and phosphorus in of soils irrigated with wastewater are interpreted compared to control: at depth of 0-15 cm of contaminated

soils,Pb is 5/6 times, Cu is 5/8 times, Cd is 23 times, Ni is 1/1 times, Zn is 25 times and P is 8/5 times and at depth of 15-30 cm of contaminated soils, Pb is 4/5 times, Cu is 5 times, Cd is 18/2 times, Ni is 1/1 times, Zn is 19/7 times and P is 8 times control soils that According to the results in amount of nickel contaminated soils, not seen a significant change compared to the control.

In wheat fields have been irrigated with wastewater compared to the control farms, the amount of heavy elements are as follows: at the depth 0-15 cm of soils contaminated farms, Pb is 1/3 times, Cu is 1/9 times, Cd is 1/3 times, Ni 0s 3/3 times and P is 3/5 times and at the depth 15-30 cm, Pb is 1/2 times, Cu is 3 times, Cd and Ni haven't changed very much, Zn is 2/8 times, P is 1/6 time compared to control farms.

Amount of heavy metals including Cd, Pb and Ni in seed corn, barley and wheat have been reported negligible Based on the results laboratory and the amount of P, Zn and Cu is normal. Therefore, it follows that the above elements haven't gathered in studied seed plant and them move in the plant. Toward seed is very slow and in comparison the pollution treatment and control is not observed a significant change.

DISCUSSION

The concentration of heavy metals Pb, Cu, Cd and Zn and P in soils irrigated with wastewater is Several times the amount of these elements in the soil the control fields that these results are match with research that Rahmani (2005) carried out on Examination of the quality urbanand industrial wastewater. The amount of soil salinity in fields irrigated with wastewater is about 2 times the salinity soil of the control fields that this match with Research results Hosseinpoor et al. (2005).

Given that one of the main sources of heavy metals are chemical fertilizers, Therefore, the use of these fertilizers should be done By studying and exact amount. Wastewater the industrial cities due to having large amounts of salts and unnecessary and heavy metals should be used with sufficient accuracy And in this regard should be conducted controlled trials. Not use for agricultural products from untreated sewage and dangerous industrial effluents.

Industrial and urbanwaste water be treated In terms of chemical and biological materials. In order to protect the environment and nutrition and development of water resources for irrigation farms is necessary that Consumption Urbanand industrial wastewater be done after filtration and removal of salt and nutrients and harmful substances from that.

REFERENCES

1. Aphioni, M., E. RezaeiNehad. 1998. The effect of sewage sludge on yield and uptake of heavy metals by lettuce and spinach. Agricultural Sciences and Natural Resources. 19: 1-30.
2. Asano, T., and J.A. Cotruvo. 2004. Groundwater recharge with reclamedurbanwaste: health and regulatory condsideration. Water Research. 38-1941-1951.
3. Carey, J.E. 1987. Metal movement in sludge emended soils: Anine- year study. Soil Sci. 143:124-131.
4. Change, A.C. 1984. Accumulation of heavy metal in sewage-sludge treated soil. J. Environ. Qual. B: 87-90.
5. Elliot, L.F. and F.J. Stevenson. 1986. Soils for management of organic waste and wastewater, second printing, Soil. Sci. Am. Inc. Publisher, Madison, Wisconsin, USA, PP. 650.
6. Haruvy, N, 1997. Agricultural reuse of wastewater: nation-wide Cost-benefit analysis. Agriculture, Ecosystem and Environment. 66:113-119.

7. JafarzadehHaghighi, N. 2005. Investigation The effect of using wastewater After irrigation of cropsOn increase in the concentration of heavy metals in soil and some products. Articles Collections of Congress soil and water issues in the country.
8. Khaiambashi, B. 1997. The effect of use from sewage sludge as fertilizer in accumulation of heavy metals in soil and in plants. Master Thesis Soil Science, Faculty of Agriculture, Isfahan University.
9. Page. A.L. 1995. Land appilication of urbansewage sludge: guide lines trace elements. P. 154-181 second international symposium on land application of sewage sludge. Tokoyo. Japan.
10. Page. A.L.1974. fate and effects of tracerments in sewage sludge when applied to agricultural land use. Environment. Prot. Agency. Cincinnati. Ohio.
11. Rahmani, H.R. 2005. Examination of the qualityUrban and industrial wastewater and their effects on soil, water and plants in Iran. Ninth Congress of Soil Science.
12. Rose. S. M. 1996. Toxic metals in soil-plant system. John wiley and sons ltd.
13. Sidle, R. C. 1977. Accumulation of heavy metals in soils as infuened by extended waste water application of sewage sludge. Irrigation. J. water pollutes. Controfed. 49:311-318.
14. Torabian, E., A. baghori. 1996. Investigation pollution by the use of urbanand industrial wastewater in agricultural land in south Tehran, the journal Ecology. Year 2. No. 18. pp: 33-45.

Table 1. The concentration of some from Heavy metals in soil and soil solution

Element	Concentration in the soil solution (toxic soils) (mg/Lit)	Average rate (Mic gr/gr)	The concentration in toxic soils (Total) (Micgr/gr)	The amount of balance from total elements in the soil (Micgr/gr)
Cr	0/001	100	75-100	5-1000
Mn	0/1-10	600	1500-3000	200-2000
Co	0/01	8	25-50	1-70
Ni	0/05	40	100	10-1000
Cu	0/03-0/3	30	60-125	2-100
Zn	<0/005	50	70-400	10-300
Cd	0/001	0/06	3-8	0/01-7
Sn	Nd	10	50	<5
Hg	0/001	0/03	0/3-5	0/02-0/2
Pb	0/001	10	100-400	2-200

Table 2. Average the soil chemical properties of corn field

Name of treatment	Depth (cm)	pH	EXC 103	OC (%)	Pb $mgkg^{-1}$	Cu $mgkg^{-1}$	Cd $mgkg^{-1}$	Ni $mgkg^{-1}$	Zn $mgkg^{-1}$	P $mgkg^{-1}$
Control	0-15	7/75	0/8	1/12	7/5	1/6	0/13	0/33	1/09	19/5
	15-30	7/75	0/8	1/15	7/5	1/45	0/13	0/31	1/29	18/5
Contaminati on with the wastewater	0-15	7/2	1/76	1/62	42/1	9/3	2/99	0/35	27/1	165/2
	15-30	7/4	1/85	1/33	33/9	7/36	2/36	0/33	25/4	150/3

Table 3. Average the soil chemical properties of barely field

Name of treatment	Depth (cm)	pH	EXC 103	OC (%)	Pb $mgkg^{-1}$	Cu $mgkg^{-1}$	Cd $mgkg^{-1}$	Ni $mgkg^{-1}$	Zn $mgkg^{-1}$	Pb $mgkg^{-1}$
Control	0-15	7/65	0/8	0/75	7/5	1/15	0/11	0/31	0/66	58
	15-30	7/5	0/7	0/7	7/75	1/15	0/11	0/48	0/58	34
Contamination with the wastewater	0-15	7	3/17	1/67	22/4	10/9	0/34	3/72	38/4	275/5
	15-30	7/12	2/95	1/35	45/3	10/8	0/34	4/35	28/1	272/2

Table 4. Average the soil chemical properties of wheat field

Name of treatment	Depth (cm)	pH	EXC 103	OC (%)	Pb $mgkg^{-1}$	Cu $mgkg^{-1}$	Cd $mgkg^{-1}$	Ni $mgkg^{-1}$	Zn $mgkg^{-1}$	Pb $mgkg^{-1}$
Control	0-15	8/1	1/13	0/51	2/55	1/88	0/17	0/22	1/08	8/46
	15-30	8/1	1/75	0/58	2/6	1/18	0/22	0/42	0/72	14/14
Contamination with the wastewater	0-15	8	1/7	0/64	3/15	3/58	0/22	0/73	2/44	29/3
	15-30	8	1/26	0/5	2/95	3/58	0/19	0/48	2/05	22/48

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Sociological Study of Satellite Effect (GEM TV Channels) on Life Style of the Female Students of the Letters Faculty of Islamic Azad University of Kerman in 2014

Kamal Javanmard* and Hamideh Namdar Mohammadi

Department of Sociology, Kerman Branch, Islamic Azad University, Kerman, Iran.

Received: 26 Mar 2015 Revised: 23 Apr 2015 Accepted: 29 May 2015

***Address for correspondence**
Kamal Javanmard
Department of Sociology,
Kerman Branch, Islamic Azad University,
Kerman, Iran.
E-mail: Javanmardkamal@yahoo.com

ABSTRACT

Media is a unique world in history of human civilization. There has never been such a fast change within history of human life because technology of communications not only has changed the world into a small village but also modernity waves have destroyed structures of societies and have created new insights and dimensions. In this direction, change of life style is considered as one of main indices of modernity. Life style forms personal identity, personality, attitude and approaches. The main objective of the present research is to recognize the effect and function of satellite channels on life styles of female students of Letters faculty of Islamic Azad University of Kerman. After testing hypotheses, it was found that there is a significant relationship between watching a Persian language satellite channel (GEM) and precocious puberty, kind of dressing, fidelity to beliefs, traditions and values. Also this channel affects considerably life style, identity and socio-cultural capital of students.

Key words: life style, satellite, Persian language channels, identity, socio-cultural capital, Gem Channel

INTRODUCTION

Regardless of extraordinary achievements and capabilities, modern human is the neediest one during history of human being. Modern human in spite of capturing other spheres is unable to conquer itself and experts believe that the main reason is modernity and involvement in technologies of modern world. The human being not only could not know itself in this world and faces with a type of self-alienation but also it confused route of happiness and

diversion and lost its socio-cultural capital and changed it into luxurious life style. At the same time, media especially satellite channels plays important roles. Satellite channels particularly Gem Persian channel by having the soft power could influence behavioral patterns of televiewers who are mostly youths and students and gradually it has changed them such that deformation of different aspects in socio-cultural life is seen evidently.

Problem statement

In sociological terms, life style is a pattern of thoughts, feelings, cognitions and acts that create socio-personal identity of an individual. According to Giddens, life style is a more or less comprehensive set of functions used by an individual because they not only meet its' current needs but also they show others personal identity which has been chosen by the individual (Giddens, 1999:120). Followers of social science believe that the individual living in the modern world manifests his\her personal identity in his/her life style. Thereby life style is accompanied with modernity because this event requires selection, the concept which is pointless in traditional culture because in past, there was no selection in practice and past traditions and customs were pre-determined programs in which there was a kind of a hidden force but in modern world, there are various selections and choices faced by individual, the individuality that resulted from modern freedom. However, an individual who undertakes a certain life style sees other selections against his/her criteria (the same, 119) and these are selection and freedom that cause the individual to choose an informed method for his interests and priorities (Bakak, 2002:78). In this direction, consumption model is the most observable and the best index of life style such that according to David Chaney, life style is social organization of consumption (Chaney, 1999:89). He believed that life style is the way of consuming, perceiving or valuing products of material culture (the same, 71). Therefore, if life style is assumed as standards of distinction (according to Bourdieu, 1984) or different life style (according to Giddens), it will be considered as patterned consumption, perception and valuation of material and immaterial culture of human beings (Chavoshian, 2005:45). Therefore, objective indices are used to measure life style in material dimension including the consuming behaviors and objects that contain measuring units as well. Time, money, specialization and efficiency are material indices of life style and consumption patterns of objects and leisure time behaviors are immaterial indices of life style. In consumption patterns, purchase power is important in material life style and ability to identify and value is important in immaterial life style. Life style is measured by indices such as activities, interests and beliefs (Gonterveis Vade, 2002:534). However, during recent decades, concerning the increasing application of media in society, behavior, habits, beliefs and values of youths have changed and some sociologists believe that the core of such changes is satellite. Increase of some satellite Persian channels in recent few decades shows the increased televiewers of such channels in a way that these channels spread different values regardless of socio-cultural aspects of different societies and these values are in contrast with values and norms governing on societies. So, concerning that individual functionality is emphasized to form identity and personality and also different age groups and their effective roles in determination of life style, the present research attempts to study effect of Gem Persian channels on change of life style of female students of Letter faculty of Islamic Azad University of Kerman in 2014.

Research necessity and importance

Significance and importance of media caused that the followers and scholars of sociology paid special attention to cognition of media and its effects and consequences on the society. It is especially important that such media involve human life although they have captured most aspects and dimensions of human life and everywhere messages of communicational devices are heard and waves of such devices have perforated in all pores and what is called as safe privacy of human beings during the history. In addition, effect of media will not be realized in an informed and direct form rather in most cases individual is affected by its content and message unintentionally. What is made such research complex and important is how to identify this mental deformation and to measure its effects.

Research objectives

Objectives have been explained based on research subject as follows

General objective

The sociological study of satellite effect on life style of female students of Letters faculty of Islamic Azad University of Kerman in 2014.

Specific objectives

Studying the role and effect of satellite specially Gem Persian channels on personal factors and life style change of female students of Islamic Azad University of Kerman

Identifying the role and effect of satellite specially Gem Persian channels on social factors and life style change of female students of Islamic Azad University of Kerman

Determining the role and effect of satellite specially Gem Persian channels on cultural factors and life style change of female students of Islamic Azad University of Kerman

Research theoretical framework

Some of media experts have considered satellite age as a new period of media history. Since cultures have life like social events, they may undergo growth, failure and deformation over the time due to interrelation and easiness of attraction or removal of their elements. From view of Mcluhan, one of the most striking experts in media, each medium is along with one of human senses. He considered three periods in human history: period of ancient civilization, script-based civilization and e-device based civilization (Saroukhani, 2012:46). From his view, mass media are divided into hot and cool media. In other words, it seems that McLuhan believed in periodic change based on labyrinthic periods. Satellite has been outlined strictly and it has affected policy, economy, culture, ideology, interests throughout the world such that it has been effective on the consuming and behavioral patterns and has changed severely life style (the same, 62). Riesman argued that human communities are changing during three stages: the first period is tradition-directed human, the second is inner-directed human and the third is other-directed human. Form his view, other-directed human is manifested with electronic communications. Modern Communicational society needs to find several methods for commercial advertisements so called subliminal advertising. Modern human being will encounter with self-alienation by extending such methods (Saroukhani, 2014:74). In this direction, Lazarsfeld and Merton are two pioneers in communicational researches. They believed that in most cases, message of media affects strongly human beings when it completes by surrounding environment. It requires homogenous message and environmental conditions. If the message contains points that are not attracted by the environment or it is not consistent with environment or it is against beliefs and values, it will be possible that the individual is affected by the message but he/she will be confused after exiting from effects resulted from attractive message of media (the same, 131). Therefore, media has affected considerably life style of human beings. Theory of the forms of capital of Pierre Bourdieu explains different consumption patterns in horizontal levels. This theory is able to measure amount of forms of capital among those who are in one class but they are different regarding the consumption pattern. He also explained differences by confirming occupational culture. Life style is those activities that have been classified by a specific method and resulted from special perceptions. In life style, individual preferences are shaped, done in practice and they are observable (the same, 45). On the other hand, Giddens believed that life styles people choose for them not only meet their current requirements but also they shape certain narratives they have chosen for their personal identity (Giddens, 1999:120). According to David Chaney, life style is a patterned

consumption. Such patterns distinguish people from each other and in modern developed societies; they are the only way for social classification (Chaney, 1999:61). From Chaney view, styles should be identified in certain contexts of lives meaning that specific interests are from certain context of personal lives as mentioned by Bourdieu (Bayley, 1997:71). However, Peterson believed that elitism has been removed from cultural consumption. Peterson argued that one of its reasons is social mobility of the modern world where lower social classes have ascended into higher classes (Peterson, 1983:425). By this model, Peterson concluded that there was no strong relationship between social class and cultural life style. On his view, tastes and interest of beautiful arts are important indices in basic distinction of people (Fazeli, 2008:91). Theory of Douglas Holt, one of poststructuralists in consumption and life style, is value- life style in which cultural mind of the individual that shapes tastes, values, preferences and interests affects his selections and choices. To explain his qualitative plan for measuring life style, Holt believes that human being has a tool which is discourse and personal relation with others (Holt, 1997:358-32).

Research hypotheses

It seems that there is a relationship between watching Gem Persian channels and change of life style of students.
It seems that there is a relationship between hours of watching Gem series and change of life style of students.
It seems that there is a relationship between watching Gem Persian channels and personal factors of students.
It seems that there is a relationship between watching Gem Persian channels and association of family members.
It seems that there is a relationship between watching Gem Persian channels and change of norms and standards of the society
It seems that there is a relationship between watching Gem Persian channels and aggression and inconsistency of students.
It seems that there is a relationship between watching Gem Persian channels and precocious puberty.
It seems that there is a relationship between watching Gem Persian channels and type of dressing
It seems that there is a relationship between watching Gem Persian channels and change of religious beliefs and traditions.

Research methodology

This research has been done by survey method and data were gathered by the questionnaire. Then data were recorded via SPSS version 21 and were analyzed by descriptive and inferential statistics. In descriptive section, statistic analyses (frequency, percentage, mean) were shown by diagrams and tables and in inferential section, analyses were tested by t-test which is the most suitable method. For other hypotheses, Pearson and Spearman's correlation coefficients have been used. The regression analysis has been applied to study the relationship between variables and prediction of their effects on the dependent variable.

Statistical population and sample

Statistical population includes female students of Letters faculty of Islamic Azad university of Kerman in 2014-2015. According to report of information & statistic center of the university, the number of students was 8233. Therefore, 370 students have been chosen by Cochran's standard formula. In present research, stratified sampling has been used and each members of statistical population has an equal chance for participation in sample population. At first, the number of female students in each field of study was measured in Letters faculty of Islamic Azad university of Kerman and the questionnaire was distributed among them randomly.

RESEARCH FINDINGS

Statistics show that 95.7% of students were single and 4.3% of them were married. 23% of respondents had associate degree, 44.9% had bachelor degree, 21.4% had master degree and 10.8% had doctorate degree. 95.4% of students were Shiite and 4.6% were Sunni. 94.6% of students used satellite and 5.4% did not used satellite. 45.9% of respondents watch Gem TV for one hour, 54.1% of them watch movies of Gem TV less than one hour. In addition, 47% of students are affected by Gem advertisements and 53% of them are not affected by Gem advertisements. Research findings suggest that regarding the role of education of students for being affected by Gem movies, 70% of respondents selected "completely agree" choice, 23.5% selected "agree" choice, 3% selected " no idea" choice and 1.9% selected "disagree" choice and 1.6% selected "completely disagree" choice. Then, respondents stated that they have less time to spend in social groups due to watching Gem series. 23.2% of respondents selected "completely agree" choice, 25.7% selected "agree" choice, 21.6% selected " no idea" choice and 20% selected "disagree" choice and 9.5% selected "completely disagree" choice. The present study suggests that due to watching Gem series, respondents have different ideas with their parents in many issues. 38.9% of respondents selected "completely agree" choice, 38.6% selected "agree" choice, 16.2% selected " no idea" choice and 4.9% selected "disagree" choice and 1.4% selected "completely disagree" choice. Also respondents believe that a number of norms and patterns of the society should be changed so 58.4% of respondents selected "completely agree" choice, 28.1% selected "agree" choice, 9.5% selected " no idea" choice and 1.9% selected "disagree" choice and 2.2% selected "completely disagree" choice. Research findings indicate that respondents believe that family members are aggressive to each other due to watching Gem series so 60% of respondents selected "completely agree" choice, 27% selected "agree" choice, 5.7% selected " no idea" choice and 3.8% selected "disagree" choice and 3.5% selected "completely disagree" choice. Moreover, respondents believe that one of factors of precocious puberty among girls is watching Gem series so 49.2% of respondents selected "completely agree" choice, 34.1% selected "agree" choice, 14.1% selected " no idea" choice and 1.9 % selected "disagree" choice and 0.8 % selected "completely disagree" choice. According to respondents, by watching Gem movies, girls tend to dress like actresses of Gem series so 58.4% of respondents selected "completely agree" choice, 27% selected "agree" choice, 6.5 % selected " no idea" choice and 4.9 % selected "disagree" choice and 3.2 % selected "completely disagree" choice. Also respondents believe that by watching Gem series, they spend some of their money on buying luxurious and expensive goods so 51.9 % of respondents selected "completely agree" choice, 32.7% selected "agree" choice, 11.9% selected " no idea" choice and 1.9 % selected "disagree" choice and 1.6 % selected "completely disagree" choice. Respondents believe that by watching Gem series, they want religious beliefs to be changed so 56.8 % of respondents selected "completely agree" choice, 26.8 % selected "agree" choice, 8.1% selected " no idea" choice and 5.1 % selected "disagree" choice and 3.2% selected "completely disagree" choice.

Regressive results of factors affecting change of life style

Table of the multi-correlation coefficient, determination coefficient, adjusted determination coefficient, estimation standard error, Durbin-Watson's test statistic

Table of regression variance analysis for studying the linear relationship between two variables

Statistical hypotheses of significance test of total regression model

H0: there is no linear relationship between two variables

H1: there is linear relationship between two variables

Analysis

Since significance level is less than 5%, the linear relationship between variables is accepted and total validity of the model is confirmed

Regressive results of personal factors affecting change of life style

Table of multi-correlation coefficient, determination coefficient, adjusted determination coefficient, estimation standard error, Durbin-Watson's test statistic

Table of regression variance analysis for studying the linear relationship between two variables

statistical hypotheses of significance test of total regression model

H0: there is no linear relationship between two variables

H1: there is linear relationship between two variables

Analysis

Since significance level is less than 5%, the linear relationship between variables is accepted and total validity of the model is confirmed

Regressive results of social factors affecting change of life style

Table of multi-correlation coefficient, determination coefficient, adjusted determination coefficient, estimation standard error, Durbin-Watson's test statistic

Table of regression variance analysis for studying the linear relationship between two variables

statistical hypotheses of significance test of total regression model

H0: there is no linear relationship between two variables

H1: there is linear relationship between two variables

Analysis

Since significance level is less than 5%, the linear relationship between variables is accepted and total validity of the model is confirmed

Regressive results of cultural factors affecting change of life style

Table of multi-correlation coefficient, determination coefficient, adjusted determination coefficient, estimation standard error, Durbin-Watson's test statistic

Table of regression variance analysis for studying the linear relationship between two variables

statistical hypotheses of significance test of total regression model

H0: there is no linear relationship between two variables

H1: there is linear relationship between two variables

Analysis

Since significance level is less than 5%, the linear relationship between variables is accepted and total validity of the model is confirmed

Inferential findings

According to statistical tests, all hypotheses have been studied as follows:

There is a relationship between social factors and change of life style of students: H1

Based on SPSS output and amount of test statistic, Pearson correlation coefficient is 0.855 with significance level of 0.000. Concerning 5% error level and comparing significance levels, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between social factors and change of life style of students with 95% confidence level.

There is a relationship between cultural factors and change of life style of students: H1

Based on SPSS output, amount of test statistic, Pearson correlation coefficient is 0.952 with significance level of 0.000. Concerning 5% error level and comparing significance levels, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between cultural factors and change of life style of students with 95% confidence level.

There is a relationship between hours of watching Gem channel and being affected by Gem series and change of life style of students: H1

Based on SPSS output, amount of test statistic, Pearson correlation coefficient is 0.132 with significance level of 0.011. Concerning 5% error level and comparing significance level, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between hours of watching Gem channel and being affected by Gem series and change of life style of students.

There is a relationship between reinforcing association of family members of students and being affected by Gem series and change of life style of students.

Based on SPSS output, amount of test statistic, Pearson correlation coefficient is 0.625 with significance level of 0.000. Concerning 5% error level and comparing significance levels, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between reinforcing association of family members of students and being affected by Gem series and change of life style of students. there is a relationship between social

norms and standards and change of life style of female students of Letter faculty of Islamic Azad university of Kerman: H1

Based on SPSS output, amount of test statistic, Pearson correlation coefficient is 0.718 with significance level of 0.000. Concerning 5% error level and comparing significance levels, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between social norms and standards and change of life style of female students of Letter faculty of Islamic Azad university of Kerman. there is a relationship between conflict and aggression and change of life style of female students of Letter faculty of Islamic Azad university of Kerman: H1

Based on SPSS output, amount of test statistic, Pearson correlation coefficient is 0.880 with significance level of 0.000. Concerning 5% error level and comparing significance levels, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between conflict and aggression and change of life style of female students of Letter faculty of Islamic Azad university of Kerman.

There is a relationship between precocious puberty and change of life style of female students of Letter faculty of Islamic Azad university of Kerman.

Based on SPSS output and amount of test statistic, Pearson correlation coefficient is 0.851 with significance level of 0.000. Concerning 5% error level and comparing significance level, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between early puberty and change of life style of female students of Letter faculty of Islamic Azad university of Kerman.

There is a relationship between kind of dressing and appearance and change of life style of female students of Letter faculty of Islamic Azad university of Kerman.

Based on SPSS output, amount of test statistic, Pearson correlation coefficient is 0.708 with significance level of 0.000. Concerning 5% error level and comparing significance level, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between kind of dressing and appearance and change of life style of female students of Letter faculty of Islamic Azad university of Kerman.

There is a relationship between tradition and religion and change of life style of female students of Letter faculty of Islamic Azad university of Kerman.

Based on SPSS output, amount of test statistic, Pearson correlation coefficient is 0.662 with significance level of 0.000. Concerning 5% error level and comparing significance level, H0 is rejected because significance level is less than 0.05. In other words, there is a positive linear relationship between tradition and religion and change of life style of female students of Letter faculty of Islamic Azad university of Kerman.

RESULTS AND DISCUSSION

According to research findings, it can be concluded that the most common variables affecting life style of students are as follows (in terms of amount of correlation): cultural factors with Beta weight of 0.639, social factors with beta weight of 0.416, personal factors with beta weight of 0.149. On this basis, the most common variables affecting personal factors of life style change of students are as follows (in terms of amount of correlation): married status with beta weight of 0.165, place of birth with beta weight of 0.99, hours of watching with beta weight of 0.87, job status with beta weight of 0.87, age with beta weight of 0.073, education with beta weight of 0.034, income with beta weight

of 0.034 and number of family members with beta weight of 0.006. in this direction, the most common variables affecting social factors of life style are as follows (in terms of amount of correlation): conflict and aggression with beta weight of 0.535, reinforcing family association with beta weight of 0.287, social solidarity with beta weight of 0.196, social alienation with beta weight of 0.174, generation gap with beta weight of 0.147, finding friend with beta weight of 0.108, norms with beta weight of 0.097, social participation with beta weight of 0.035. Also the most common variables affecting cultural factors of life style are as follows (in terms of amount of correlation): kind of dressing with beta weight of 0.318, precocious puberty with beta weight of 0.312, fashion with beta weight of 0.127, traditions with beta weight of 0.125, tendency to make-up with beta weight of 0.108, luxuriant living and diversity with beta weight of 0.097, consumerism with beta weight of 0,070, spirit of mimicry with beta weight of 0.023.

Research suggestions

Undoubtedly, the role of media has been effective on life style and it can be said that the function of most media is to change gradually life style of human beings. Therefore, an Islamic and Iranian life style should be seek in order to institutionalize it in the society and to come over against the soft war and cultural attacks. role of women as the key element of changing life style of the family into an Islamic and Iranian style is inevitable so this type of life style should be internalized in women. domestic media especially national medium should explore scientifically and accurately huge effects and changes in global scale especially satellite Persian series and they should predict future events in short and middle terms by taking into account a future-based research approach and by knowing such effects and changes and media should be matched with them before west culture destroys traditions, values and culture.

Penetration factor of national medium should be increased in society especially in Kerman city.

Executive strategies

1. Since Iran is an Islamic society, media should spread this kind of life style and one of ways for spreading Islamic life style is virtual space and this type of life style can be taught via web sites, weblogs, on line advices, chat and so on.
2. By introducing superior patterns and picturing Islamic successful families, media can spread Islamic life styleMedia by criticizing west life style can illustrate and clarify harmful components of such life style and its negative consequences
3. By holding educational workshops, classes and practical credits for Islamic life style among women, correct life style and modification of consumption pattern of the family (that has been toward consumerism), the families can be directed into tranquility and enjoying from life via training them to be thrifty
4. In this direction, increasing knowledge and cognition of social members and informing them from components of Islamic life style should be attempted by national media such that they should re-identify socio-cultural capital and identity by increasing media literacy of people, making TV movies and historical series using national heroes in different fields.
5. Diversification of fashion in Islamic form with maintaining social norms and values
6. Formulating programs based on reinforcing components of Islamic life style in form of cultural engineering especially in domestic media is considered as a step towards reduction of effect of global media on life style of students

REFERENCES

1. Aron Raymond (2007), basic stages of thinking way to sociology, translated by Bagher Parham, Tehran, cultural-scientific publication
2. Khademian, Talieh (2012), life style and cultural consumption, publication of artistic-cultural institute of Jahan-e Ketab
3. Davis Charles (2008), religion and construction of the society, inquiries in social theology, translated by Hasan Mohadesi and Hossein Babolhavaeji, Tehran, Yadavaran publication
4. Saroukhani, Bagher (2014), sociology of communications of principles and basics, Etela'at publication
5. Saroukhani, Bagher (2012), modern sociology of communications of media in today world, Etela'at publication
6. Sarajzadeh Hossein (2005), challenges of religion and modernity, sociological issues in religion and secularism, Tarh-e No publication
7. Ian Craib (2014), modern social theories from Parsons to Habermas, translated by Abbas Mokhber, Tehran, Agah publication
8. Bruce Cohen (2012), sociological basics, translated by Mohsen Salasi, Tehran, Farhang-e Moaser publication
9. Anthony Giddens (2013) consequences of modernity, translated by Mohsen Salasi, Tehran, Markaz publication
10. Anthony Giddens (2014), modernization and individuation of society and personal identity in modern age, translated by Naser Movafaghian, Tehran, Nei publication
11. Quarterlies and theses
12. Fazeli, Mohammad, picture of cultural life style of students, quarterly of cultural researches, first year, No 1
13. Mahdavi Keni, Mohammad Saeed (2008), concept of life style and its scope in social science, research-scientific quarterly of cultural researches of Iran, first year, No:1, Pp 199-230
14. Kizghan, Iman (2003), studying factors affecting social identity of women: case study of Shiraz city, Alzahra humanities faculty, winter 2002 and spring 2003, Nos:12 and 13, Pp 79-107

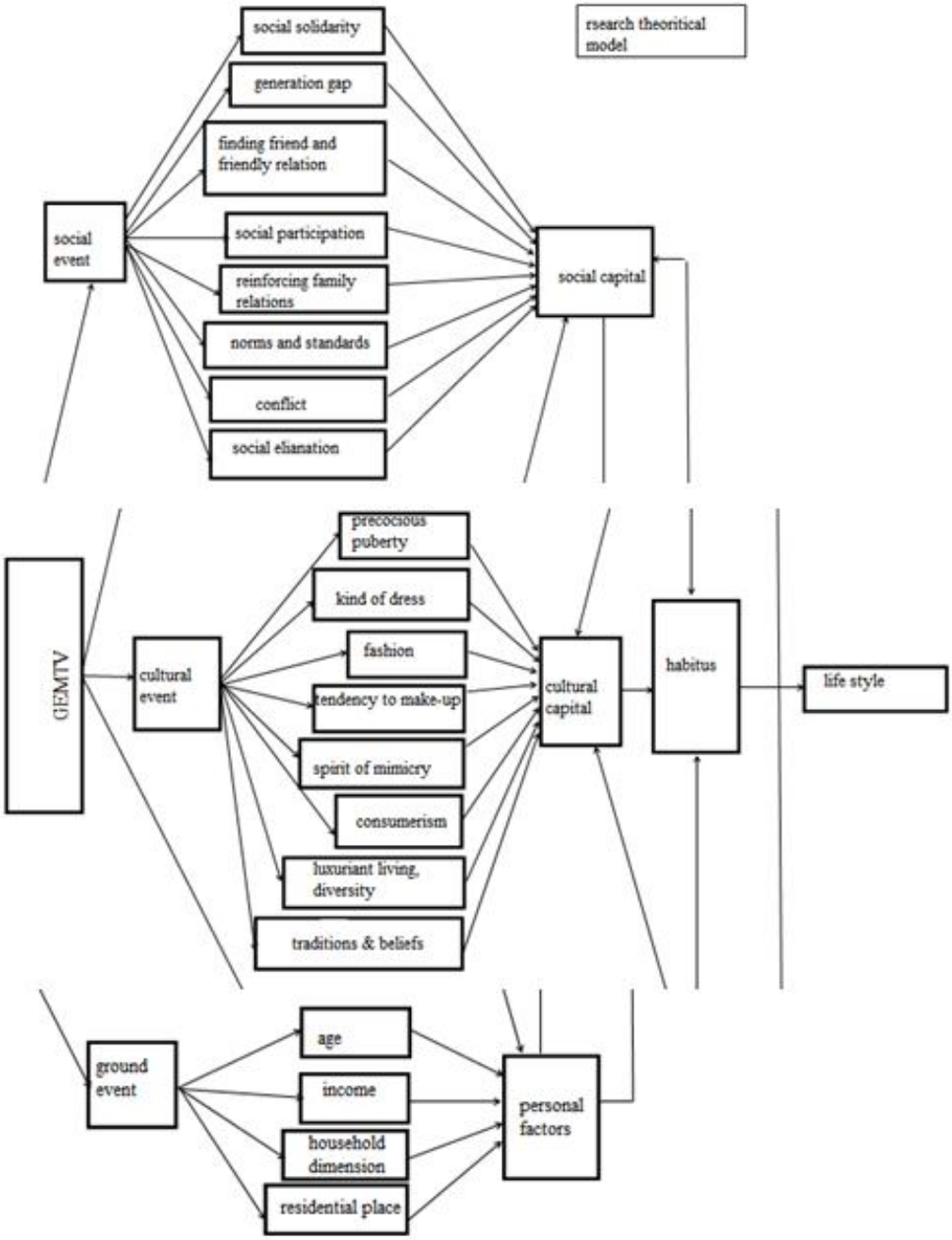
rsearch theoritical model
social solidarity
generation gap
finding friend and friendly relation
social participation
reinforcing family relations
norms and standards
conflict
social elianation
social event
social capital
GEMTV
cultural event
precocious puberty
kind of dress
fashion
tendency to make-up
spirit of mimicry
consumerism
luxuriant living, diversity
traditions & beliefs
cultural capital
habitus
life style
ground event
age
income
household dimension
residential place
personal factors

Fig:1

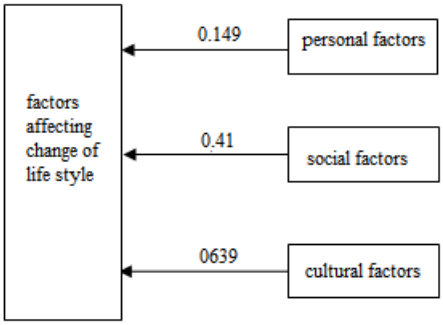

Fig: 2

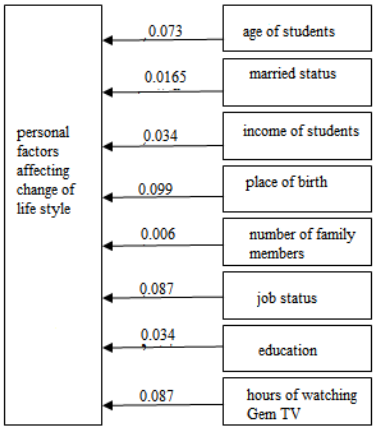

Fig: 3

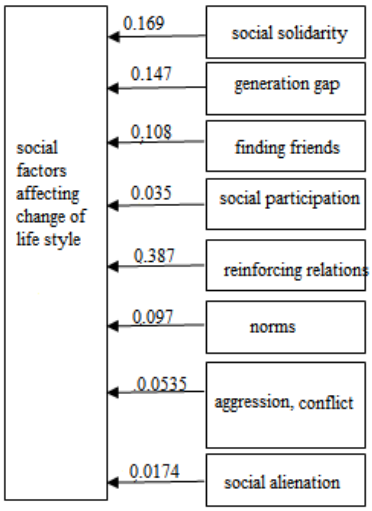

Fig: 4

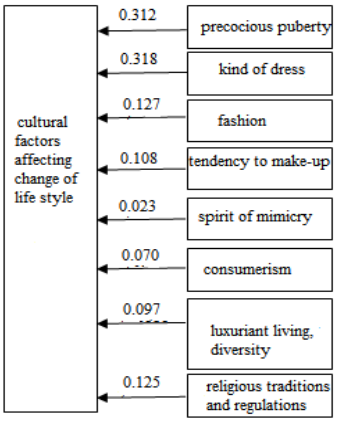

Fig: 5

Table of theories' matrix

row	Field	Name of theorist	Name of theory	Basic indications and variables
1	sociology	Emile Durkheim	functionalism	Holy and unholy, collective services, group interdependence, coherent functions, discipline, totem and taboo
2	sociology	Karl Marx	Class conflict, contradictions of capitalism system	Consciousness, emotional area, governing class, capitalism
3	sociology	Max Weber	Life style	Life conducts, life opportunities (age, class, gender, ethnicity)
4	sociology	David Chaney	Social organization of consumption	Tastes, initiatives, feelings, levels
5	sociology	Talcott Parsons	Structuralism	Rules, norms, patterns
6	sociology	Thorstein Veblen	Leisure class	Conspicuous consumption and leisure, leisure class
7	sociology	George Zimmer	Consumption	Internal motivations, personal factors, status symbols (being distinct, personal identity), group correlation, social solidarity
8	sociology	Anthony Giddens	Identity, consumption sociology	How to consume, type of good, limitation, opportunities
9	sociology	Pierre Bourdieu	Habitus, practice theory	Taste, behavior (economic capital, social capital, field)
10	sociology	Peterson	Hybrid cultural style	Taste, initiative, beautiful arts
11	sociology	Douglas Holt	Value- life style	Tastes, values, priorities, interests

Regressive results of factors affecting change of life style

model	R	R square	Adjusted R square	Standard error of the estimate
1	.999 [a]	.999	.999	.51250

Table of regression variance analysis for studying the linear relationship between two variables

model	Sum of squares	df	Mean square	F	Sig.
Regression	92980.844	3	30993.615	001.839	.000
Residual	96.131	366	.263		
total	93076.976	369			

Table of coefficient of regression equation and significance test of these coefficients

model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
(constant)	.608	.224		2.719	.007
Personal factor	.987	.012	.149	85.848	.000
Social factors	.987	.006	.416	173.941	.000
Cultural factor	1.059	.004	.639	263.592	.000

Regressive results of personal factors affecting change of life style

model	R	R square	Adjusted R square	Standard error of the estimate
1	.259 [a]	.067	.047	15.50779

Table of regression variance analysis for studying the linear relationship between two variables

model	Sum of squares	df	Mean square	F	Sig.
Regression	6259.520	8	782.440	3.254	.001
Residual	86817.455	361	240.492		
total	93076.976	369			

Table of coefficients of regression equation and significance test of these coefficients

model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
(constant)	61.152	4.592		13.316	.000
Age of students	1.268	.902	.073	1.406	.161
Married status	1.869	.578	.165	3.233	.001
Income of students	.573	.857	.034	.668	.504
Place of birth	2.009	1.053	.099	1.908	.057
Number of family members	.141	1.203	.006	.117	.907
Job status	2.847	1.748	.087	1.629	.104
Education	.969	1.476	.034	.656	.512
Hours of watching Gem TV	2.926	1.820	.087	1.608	.109

Regressive results of social factors affecting change of life style

model	R	R square	Adjusted R square	Standard error of the estimate
1	.935 [a]	.874	.871	5.70982

Table of regression variance analysis for studying the linear relationship between two variables

model	Sum of squares	df	Mean square	F	Sig.
Regression	81307.645	8	10163.456	311.743	.000
Residual	11769.331	361	32.602		
total	93076.976	369			

Table of coefficient of regression equation and significance test of these coefficients

model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
(constant)	13.140	2.273		5.780	.000
Social solidarity	1.302	.130	.196	10.002	.000
Generation gap	1.061	.139	.147	7.645	.000
Finding friend	1.582	.285	.108	5.552	.000
Social participation	.300	.232	.035	1.295	.196
Reinforcing family relations	2.879	.285	.287	10.099	.000
norms	.919	.315	.097	2.922	.004
Aggression, conflict	6.556	.567	.535	11.563	.000
Social alienation	1.464	.283	.174	5.167	.000

Regressive results of cultural factors affecting change of life style

model	R	R square	Adjusted R square	Standard error of the estimate
1	.961 [a]	.923	.922	4.44957

Table of regression variance analysis for studying the linear relationship between two variables

model	Sum of squares	df	Mean square	F	Sig.
Regression	85929.668	8	10741.208	542.5	.000
Residual	7147.308	361	19.799	23	
total	93076.976	369			

Table of coefficients of regression equation and significance test of these coefficients

model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
(constant)	36.004	1.335		26.960	.000
Precocious puberty	2.579	.256	.312	10.093	.000
Kind of dressing	2.494	.453	.318	5.506	.000
Fashion	1.083	.334	.127	3.242	.001
Tendency to make-up	1.200	.734	.108	1.635	.103
spirit of mimicry	1.707	1.300	.023	1.313	.190
consumerism	1.310	.612	.070	2.140	.033
Luxuriant living, diversity	.895	.474	.097	1.886	.060
Tradition	.930	.284	.125	3.272	.001

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Influence of Metacognitive Training on Decrease of High School Teenagers' Stress

Lila Barjasteh[1] and Vida Razavi[2]*

[1]M.A.student in Psychology, Department of Psychology, Science and Research Branch, Islamic Azad University, Yazd, Iran.
[2]Department of Educational Science & Psychology, Kerman Branch, Islamic Azad University, Kerman, Iran.

Received: 20 Mar 2015 Revised: 19 Apr 2015 Accepted: 28 May 2015

***Address for correspondence**
Vida Razavi
Department of Educational Science & psychology,
Kerman branch, Islamic Azad University,
Kerman, Iran.
E-mail: vidarazavi2010@yahoo.com

ABSTRACT

The metacognitive skills help the person to choose the methods and proper solutions when confronting problems and stop their ongoing procedure and control the situations that can be harmful by a proper planning. This research aimed to investigate the influence of metacognitive skills training on decrease of teenagers' stress. This research was of semi-experimental type and was implemented with pre-test – interference – post-test pattern. To do so, first through a multistep random sampling in different Education districts of Kerman Province, one district (District 1) was selected and then six high schools were chosen and 600 students were sat to take the stress test. After scoring, 60 out of 120 students who had the highest score of stress test were selected and were divided to two 30-persons groups. The test group included 30 students including 15 boys and 15 girls, and 30 other were the control group. Then the test group members received metacognitive training through 8 two-hour sessions, but the control group did not receive any training. At the end, both groups were compared by stress test. To analyze data, the covariance analysis methods and T test were applied and the findings showed that the metacognitive trainings were influential on decrease of teenagers' stress.

Key words: Stress, cognition, metacognition.

INTRODUCTION

Stress or mental pressure is a reaction towards situations in which the person must meet expectations that are confronted with limits and barriers and must take chances. [3].

Stress is the widespread mental disease in the world today. Because of the pain and hurt that the suffering people endure, as well as the big burden caused by this problem on nations' therapeutic resources, importance of this disease has been noted more than ever during the recent two decades [1]. Stress is one of the most prevalent type of mental diseases that make patients refer to psychiatrists and psychologists and other mental health experts. [20].

Stress is a set of physical, mental, psychic and behavioral reactions that human's organism (or animals') shows towards internal or external stimulus (factors) disrupting stability and natural balance and internal body. The major aim of these reactions, is returning the lost balance of organism and person's compatibility with the environment. [13]

Stress is an inevitable phenomenon. Regardless of gender, age, social class, wealth, career and time, it is always present. Stress has always been mentioned as one of the most important factors of appearance and formation of various physical and mental diseases and death of people, and since numerous effects of stress are physiological, it is therefore considered a bio-psychological disorder. [10].

Stress is derived from dynamic interaction between the person and environment. People experience stress, when they see their needs and demands are in contrast with the environment. [13]. A number of scientists, especially British researchers who have done numerous researches, introduce stress and mental pressure as the reason of many physical diseases (like, ulcer, cardiovascular, strokes, blood pressure and some joint paints, etc.) [16].

People's evaluation of stress and their dealing and facing it is very important. Stress would be usually harmful when the person finds it dangerous and threatening and at the same time lack various resources of encountering it. Researches show that applying effective encounter strategies has an important role in decrease of stress [11].

In the meantime, cognition is known as "internal mental processes or how data are processed, i.e., the methods by which we pay attention to data and codify them and keep in mind and recall and use them whenever we need". [2]

In other words, we know our surrounding world through cognitive processes and become aware of it and respond to it. Cognition refers to processes by help of which people learn to think and remember. In brief, cognition means knowing and perception of cosmos, knowing the cosmos. The term metacognition is also suggested as our knowledge about our cognition processes and the way of optimized application of them for reaching to learning goals. Metacognition is person's knowledge or awareness of self-cognition system or is the knowledge of knowledge. This knowledge helps us consider our progress while learning and knowing things. [26].

Metacognition is our knowledge of our cognition processes and its optimized application to get to the goals. The metacognition knowledge helps us monitor our progress while learning about different things and doing homework. This knowledge helps us assess results of our efforts and evaluate our dominance. Metacognition is our knowledge of self-cognition system and how to control it. Metacognition is a cognition beyond ordinary thinking and is applicable to one's knowledge of cognition, learning and way of thinking. Metacognition refers to knowledge of structures and psychological processes which are dealing with interpretation and rendition of thoughts and cognitions and is one of the important factors of development and continuity of psychological disorders. So if we consider this cognition as knowing and learning, we can consider metacognition as knowing about the way we learn and think. [23].

Teenagers' knowledge of their stress helps them and develops their knowledge of the harms, damages and results and prevents stress from developing. Today the importance of metacognition is accepted for higher levels learnings. Learners can enjoy maximum successful learning when they have a good knowledge of their personal ability. If teenagers learn about the harms and destructive role of stress in life and have sufficient information about it, they will be able to cope with this problem and apply the preventive manner and way of confronting it. [15]. Therefore this research investigates the influence of metacognitive skills training in reducing teenagers' stress.

METHODS

Present research was of semi-experimental plot type. Collected information this research were obtained through pre-test-interference-post-test method. To investigate the influence of independent variable (metacognitive skills) on dependent variable (stress level), covariance analysis and T test methods are applied.

Statistical population of this research includes all high school students of Kerman and out of all districts, one single one (District number 1) and of this district six schools (three girls school and three boys school) were selected through multistep cluster random sampling. First, 600 girls and boys sat to take stress test. Then after scoring, 60 out of 120 students who had the high scores were selected and were divided to test and control groups (15 girls and 15 boys). They received metacognitive trainings in 8 sessions each for two hours, but the control group did not receive any training.

Research tool

Cohen stress questionnaire.

This questionnaire was prepared by Cohen et all back in 1983. It consists of 3 copies of 14, 10, 4 clauses. They were used for general stress assessment through the past one month. It investigates thoughts and emotions about stressful events, control, dominance, getting along with mental pressure and shows the process of tense relations.

Scoring questionnaire is on the basis of 5-degree Likert scale: never=0, almost never=1, sometimes=2, often=3, most of the times=4. Clauses 4-5-6-7-9-10-13 are oppositely scored (never=4, most of the times=0). The lowest score is 0 and the highest is 56. Higher score shows the highest perceived stress. According to Cronbach alpha, stability of this test in three studies are 84%, 85%, and 86% (Cohen et alll., 1983).

For its stability in Dr.Gholamreza Bash and Bozorgian research in two methods of Cronbach alpha bisection of results are equal to 84% and 81%.

FINDINGS

To investigate the influence of metacognitive skills training on decrease of girl and boy teenagers, the covariance test was used. Of course it must be mentioned that to investigate this test, two default points had to be considered. Before assessing the test, the homogeneity of regressions, as well as linear relationship in regression level of the test and control groups were investigated. In the next stage, Levin test was used to check the homogeneity of variances of the two groups.

Main hypothesis of the research Metacognitive training is influential on decrease of teenagers' stress

Investigation of Levin test results showed that the significance level of P=0.198 is higher than significance level of 0.05, so the assumed homogeneity of variances is confirmed and the variance of dependent variable error among the test and control groups is equal.

By considering the scores of pre-test stress variable as an auxiliary in covariance analysis, the post-test scores of the test and control groups were compared. Statistically, the influence of pre-test scores was significant in post-test scores of the teenagers. In other words, after adjusting the pre-test scores, there was a significant influence of factor between the subjects of the group (F=37.588, P<0.05).

Secondary hypothesis

First hypothesis Metacognitive training is influential on decrease of teenage boys' stress

Investigation of Levin test results for error variances equality showed that the assumed homogeneity of variances is confirmed and the variance of dependent variable error among the test and control groups is equal.

By considering the scores of pre-test stress variable as an auxiliary in covariance analysis, the post-test scores of the test and control groups were compared. Statistically, the influence of pre-test scores was significant in post-test scores of the teenage boys. In other words, the pre-test scores affected the post-test scores and the stress level of post-test in the test and control groups is significant.

Second hypothesis Metacognitive training is influential on decrease of teenage girls' stress

Investigation of Levin test results for error variances equality showed that the assumed homogeneity of variances is confirmed and the variance of dependent variable error among the test and control groups in teenage girls is equal.

According to covariance analysis, the influence of pre-test scores was statically significant in post-test scores of the teenage 803girls in the test and control groups. In other words, the pre-test scores affected the post-test scores. That means, after girls' metacognitive training, the decrease of stress in the test group is observed.

DISCUSSION AND CONCLUSION

Stress is a set of physical, mental, psychic and behavioral reactions that human's organism (or animals') shows towards internal or external stimulus (factors) disrupting stability and natural balance and internal body. The major aim of these reactions is returning the lost balance of organism and person's compatibility with the environment. [2].

Stress is a normal part of life, because as long as the stress is not severe or prolonged, it provides the ground for growth and flourish. What is more important when facing the stress, is the way the person faces and evaluates the stressing situation. With metacognitive trainings teenagers can be protected when facing different stresses that exist in the environment and in their interpersonal relations. Adolescence is a critical period of human's life which is defined as the chaos and stress period by a number of psychologists. They believe that if teenagers finish this period without problem, they will turn to a healthy adult and will perfectly play their roles. But if they face difficulties in this period, their mental health will go astray and lose the main track. On the other hand, contrary to many evolutionary phenomenon, stress in childhood or adolescence is not a transient one which would heal through the evolution period. Rather, experiencing stress in adolescent years, would predict the strength and continuity of stress in adulthood and if the influential factors in forming them remain without intervention, the subsequent complications would also be inevitable. It must be mentioned that the stress issue in adolescence is more important

because there are numerous imbalances and crises during this period. [25]. Teenagers' stress is a serious and widespread issue. That is why knowing stress and its signs and teaching necessary skills are important factors in diagnosis and proper treatment.

Main hypothesis : Metacognitive training is influential on decrease of teenagers' stress

The obtained results of this research showed that the metacognitive training is influential on decrease of teenagers' stress. The results show that there is a significant difference between the teenagers' stress level before and after the metacognitive skills training. These results comply with findings of Koboka (2009), Fiostein et all (2000), Flowell (1988), Shonfield (1996), Motavalli (1997), Rahimpour (2005), Taalebzade (2002), Fahimzade (2002), Salehi (2004), Seif (1998). They all used cognitive techniques in treatment of stress and confirmed the role of metacognition in treatment of stress.

Secondary hypotheses

First hypothesis : Metacognitive training is influential on decrease of teenage boys' stress. The results show that there is a significant difference between the teenage boys' stress level before and after the metacognitive skills training. These results comply with findings of Moorie et all (2002), Hein et all (2005), Chen et all (2006), Oei (2008), Akhond Makkei (1997), and Gorji; Mehrabizade (1996), Sanaei and Nasiri (2000), Hanasaabzade et all (2001), Taraghijaah (2006), Taheri and Jamshidi (2007), and Nazari and Asadi (2011). They all used cognitive approach and techniques in treatment of stress and confirmed the role of metacognition in treatment of stress.

Second hypothesis :Metacognitive training is influential on decrease of teenage girls' stress

Metacognitive training is influential on decrease of teenage girls' stress. This research show that there is a significant difference between the teenage girls' stress level before and after the metacognitive skills training. These results comply with findings of Akhond Makkei and Gorji (1997), Mehrabizade (1996), Sanaei and Nasiri (2000), Hanasaabzade et all (2001), Taraghijaah (2006), Taheri and Jamshidi (2007), and Nazari and Asadi (2011) who all used cognitive approach and techniques in treatment of stress and confirmed the role of metacognition in treatment of stress. So it is recommended that the results of such researches are provided to related institutions and lead to implementation of proper solutions to decrease teenagers' stress.

REFERENCES

1. Akbari, Bahman. 1996. Investigation of relation between stress and method of confronting mental pressure. BA thesis.
2. Bieler ,R.F.& Snowman ,J. 1993. Psychology applied to teaching (1 th ed.).Hoagthon Mifflin.
3. Cooper, C.L.,& Dewe , P. 2005.Stress :A brief history. UK: Black well publishing LTs.
4. Dadsetan, Parirokh. 1997. Evolutionary psychopathology. Tehran: Samt Publications. Sixth edition.
5. Fahimzadeh, Sedighe. 2002. Investigation of influence of metacognitive strategies training on Arak university students' progress motivation. MA thesis, Tehran University: Alzahra University, Psychology and Education of Sciences College.
6. Felavel ,J. 1979.Metacognation and cognation monitoring .American psychologist.
7. Fiorestein et all. 2000. Increasing metacognitive skills. Translated by Ali Eslambolchi Moghaddam. Tehran University Publications.
8. Flowel, John H. 1988. Cognitive growth. Translator: Maher, (1998). Rosh publications.
9. Isank 1994. Stress decrease methods. Translated by Yaasaaei. Tehran University Publications.
10. Khodayarifardm Mohammad. 2006. Stress and ways to face it. Iran. Tehran University Publications.

11. Kohen , H.M. 1995.stress : liner and curvilinear Effects of psychology.International Journal of stress management.
12. Lazarus, R.S. 1993. Coping Theory and research: past,present. And future . Psychosonatic medicine
13. Lazarosen, Arnold 2001. Eclectic psychotherapy. Translators: Akbari, Shahnam Abolghasemi. Tehran. Firooze Publications.
14. Mayer, Richard, E. 1986. Educative psychology. Translator: Farahani, (1997). Tehran, Islamic Revolution documentation center.
15. Mehryar, Amir Hooshang. 1994, Stress and cognitive therapy. Roshd Publications.
16. Miller.s 1995. The relationship between junior high school teachers perceptions of healthy shool environment and their trust to principle , colleagues and the school organization doctoral dissertation .the university of Toledo
17. Motavalli, S.M. 1997. Investigation of influence of metacognitive trainings on first grade girl high school students in Ferdos town. MA thesis - Psychology and Education of Sciences College, Allame Tabatabaei University.
18. Nazari, Ali Mohammad; Asadi, Masood. (2011). Investigation of influence of group therapy on decrease of stress in students. Knowledge and Health Journal. Period 6. No.1.
19. Okhovvat, Valiollaah; Jalili, Ahmad. (1983) Stress. Rose Publications. First edition.
20. Rahimpour, Shokoofe. (2001). Investigation of metacognitive training role in solving math problems. MA thesis. Shiraz University. Education of Sciences College.
21. Salehi Fadavi, Javad. (2001). Feeling and perception psychology. Mani publications.
22. Seif, Aliakbar. (2007). Modern educational psychology. Tehran: Publications: Doran. Sixth edition.
23. Shonfield, E.H (1999). Metacognition and mathematics. Translated by Farhad Karimi, Rosh educational.
24. Soltani Amirabadi, Marzie. (2002). Investigation of relation between creativity and stress methods of facing in high school students in Isfahan. MA Thesis of General Psychology – Psychology and Education of Sciences College.
25. Talebzadeh, Hadi, (2002). Investigation of relation between knowledge of moral metacognition and reasoning and moral behavior. (MA thesis), Training University, Psychology and Education of Sciences College

Table 1- Results of Levin test about default equal variances of test and control groups in teenagers

significance level	Second degree of freedom	First degree of freedom	F	variable
0/259	58	1	1/302	Pre-test variable

Table 2- Results of analysis of influence of metacognitive training on post-test stress in teenagers

Statistic power	Eta index	p	F	Squares mean	degree of freedom	Research variables	Dependent variable
1	0/702	0/000	134/566	1062/693	1	Pre-test	Post-test
1	0/397	0/000	37/588	296/843	1	G	

Table 3 - Results of Levin test about default equal variances of test and control groups in teenage boys

significance level	Second degree of freedom	First degree of freedom	F	Gender	variable
0/591	28	1	0/295	Male	Pre-test variable

Table 4- Results of analysis of influence of metacognitive training on post-test stress in teenage boys

Statistic power	Eta index	p	F	Squares mean	degree of freedom	Research variables	Dependent variable
1	0/756	0/000	83/719	634/048	1	Pre-test	Post-test
0/941	0/331	0/001	13/387	101/385	1	G	

Table 5- Results of Levin test about default equal variances of test and control groups in teenage girls

significance level	Second degree of freedom	First degree of freedom	F	Gender	variable
0/182	28	1	1/876	female	Pre-test variable

Table 6- Results of analysis of influence of metacognitive training on post-test stress in teenage girls

Statistic power	Eta index	p	F	Squares mean	degree of freedom	Research variables	Dependent variable
1	0/639	0/000	47/078	390/803	1	Pre-test	Post-test
0/998	0/489	0/001	25/864	214/703	1	G	

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Effect of Garlic Essential Oil and Nano-encapsulated Garlic Essential Oil on the Shelf Life of Hamburger and Sensory Characteristics of Hamburger

Parisa Homayounpour[1]*, Mina Mahdavi Yekta[2], Forooghlankarani[3], Afshin Akhund Zadeh Bosti[4], Hossein Jalali[1] and Ali Khanjari[4]

[1]Department of Food Science, Damghan City Branch, Islamic Azad University, Damghan, Iran.
[2]Department of Food Science, Faculty of Agriculture's Technical, University of Tehran, Iran.
[3]Department of Food Science, Qods City Branch, Islamic Azad University, Tehran, Iran.
[4]Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Iran.

Received: 25 Mar 2015 Revised: 27 Apr 2015 Accepted: 30 May 2015

***Address for correspondence**
Parisa Homayounpour
Department of Food Science,
Damghan City Branch, Islamic Azad University,
Damghan, Iran.
Mobile: 0098(912)5659580
E-mail: h.parisa77@yahoo.com.

ABSTRACT

Hamburger is one of the most used meat products that its high nutritional value, flavor, convenient use and lack of additives have increased its consumption around the world. Microbial growth during storage of hamburger is considered an important factor in the degradation of its quality and one of the main concerns of manufacturers. In this research thyme garlic and garlic essential oil of nano-encapsulated were prepared as a natural antimicrobial compound with0,0/015% and 0/03% concentrations and were added to the formulation of 100g hamburger samples. Prepared hamburger samples were kept in 4 ° C for 12 days. Then sampling was carried out on days zero, 2nd, 4th, 8th and 12th biological .Tests (Salmonella, total count, Staphylococcus aureus and mold and yeast), organoleptic tests (flavor, odor, and color) and physical tests (cooking loss and color)were done. Tests were repeated three times on each sample Statistical analyses were carried out by SAS statistical software .To analyse the sensory tests the Kruskal-Wallis test was used given the significant differences between treatments. The Duncan test at 0/05 level was used to analyze data. Results showed that thymegarlic and garlic essential oil of nano-encapsulated have a good antimicrobial effect on hamburger and lead to microbial reduction and increased durability. In addition, the best antimicrobial effect was at 0.03% concentration compared with

0/015% and no color and odor changes were seen.The flavor was better in 0/03% concentration compared with 0.015% and control sample and no cooking losses were observed.The thyme Garlic and Garlic essential oil of nano-encapsulatedhad no significant effect on the chemical composition of the hamburger.The results indicated that the Garlic and Garlic essential oil of nano-encapsulated can be used as a natural preservative to increase the shelf-life of hamburgers and to enhance its flavor.Nano-encapsulated garlic oil and garlic oil also had a lasting effect even more.

Key words: Garlic and garlic essential oil of nano-encapsulated, Hamburger, Shelf life, Sensory Characteristics, Microbial test

INTRODUCTION

Hamburger is one of the meat products that is used by millions around the world (Hosseini et al., 1390) and due to various reasons including ease of use, use of meat in its mixture and pleasant taste has a high rate of consumption (Fernandez-Gine et al., 2005). This product is a Homogeneous mixture of meat, onion, Bread crumbs and other edible additives that is produced and supplied as an industrial or handmade product in manufacturing units of the country and supplied. According to national standard of Iran, industrial hamburgers are divided in to three groups of products containing 30% meat, ordinary hamburger, containing 60% meat, premium hamburger, and containing more than 60% of meat (Anonymous 2008). Considering the fact that hamburger up to the time of consumption is a raw product,its Microbial Quality control is necessary. Major microorganisms contaminating hamburgers are bacteria such as Staphylococci, bacilli, lactic acid bacteria and yeast such as Candida (Fernandez-Gina et al., 2005).

Anti-microbial compounds used in food products almost take on two main approaches. First, developing information about antimicrobial natural substances and second, using natural antimicrobial substances in combination with one another or traditional or new methods of processing for determining Possible synergistic effects of these compounds (Bhurinder et al., 2001).

Extensive application of natural compounds is due to their high antioxidant and antimicrobial potential. Essential oils are Volatile, natural and complex compounds and are produced by aromatic plants in secondary metabolite form (Baydar et al., 2004). Essential oils are known as ethereal or volatile oils that are considered as one of the most important natural preservatives. Around 300 species of known essential oil are available that almost 30% of them has commercial importance (Burt, 2004).

Essential oils and their compounds are considered as secondary metabolite of plants and their antibacterial properties has been known for years. They have so many usages as preservatives and flavors in food and pharmaceutical industries (Bhurinder et al., 2001; Plamer et al., 2002). Although, essential oils in food are used as antibacterial compounds and in meat products due to having a larger fat content larger amount of these compounds are needed, However, it should be heeded that their sensory characteristics are also important (Khobkar et al., 2012).

The use of natural additives as antibacterial compounds is an appropriate solution for controlling pathogenic bacteria and increasing shelf life of processed food products that reduces health threats and economic loss resulting from microorganism growth with food origin (Shaiq Ali et al., 2000). Most probably the most notable application field of essential oil is prevention of growth and also reducing the number of Pathogenic microorganisms in food. Similarly, due to delaying corruption and improving sensory quality will attract attention in commercial term (Burt, 2004). Hence, exploring the effect of essential oils or their compounds alone and together on a number of Pathogenic microbes transferred though food such as Listeria Monocytogenes, Staphylococcus aureus, Escherichia coli,

Salmonella, Shigella, Clostridium.perfringens and Bacillus cereus is so much important (Juneja et al., 2006; Chi-Zhang et al., 2004; Etlaybi et al., 2000; Misaghi and Akhondzadehbasti, 2007).

Garlic, with scientific name of Allium Sativum, is a Monocotyledonous, grass and one-year plant (Allium means penetrating odor), that belongs to "Alliaceac" family that is native of middle east and today is found in all locations of the world. Among edible plants, Alliums stadium, is known as a Miraculous medicine. Garlic has two important sub-plants, known as Ophioscordon or Hardnecked and sativum or softnecked. Various species of this plant from centuries ago have been used as spice for food as well as medicine in herbal medicine for treating various types of illnesses (3).

Garlic has antibiotic, anticancer, antioxidantproperties,and protects cardiovascular system. Medical and anti-microbial effects of garlic due to existence of organophosphorus compounds that contains 2- propenylallylsufinate as its most important constituent that is known as allicin that often medical properties of garlic are due to allicin decomposition. This compound constitutes around 1.5% of total weight of the plant and is responsible for its odor. Allicin in the fresh plant is found in the form of a precursor known as alliin that is colorless and odorless and due to fragmentation of an enzyme named alliinase with affecting alliin turned it into alicin which is the reason behind strong odor of garlic. Garlic extraction is edible and can be used in different foods and very well can transfer antibacterial and antioxidant properties of garlic (3).

Among effective compound of garlic against microorganisms, in addition to agoene, allyl methyl tri-sulfide, diallyl sulfide, dimethyl disulfide, dipropyl disulfide and methyl agoene can be mentioned. As one of the other most important known properties of garlic diallyl terry sulfide, diallyltetrasulfide, diallylpentasulfide, diallylhexasulfide and diallylheptasulfide can also be mentioned. This, specailly in meat products such as hamburger that always are prone to getting rotten as the result of lipid oxidation is so much important (3).

Anti-inflammatory properties and reduces blood sugar and

Considering the increasing consumption of meat products by people day by day, use of natural preservatives for inhibiting the growth of pathogenic bacteria is a valuable idea in the industry of meat products for the purpose of securing the health of consumers. Hence, the aim of the present study is that with the help of garlic essential oil improve sensory characteristics of the product in addition to preserving its nutritional value.

Nano-encapsulation of bioactive compounds indicates to an efficient method for increasing physical sustainability of active substances, increasing food compounds preservation and increasing their biological activity. Regarding, antibacterial compound, encapsulation can increase concentration of bioactive compounds in those parts of food in which there are more microorganisms, such as rich phases of water and solid-liquid locations. Among the other advantages of encapsulation of essential oils we can refer to protection of active compounds against environmental factors such as oxygen, light, humidity and PH (20).

MATERIALS AND METHODS

Research method

In this study garlic essential oil and nano-encapsulated garlic essential oil have been used as natural anti-microbial compound with concentrations of 0.03% and 0.015% in formulation of 100 g hamburger samples containing 60% meat and the prepared hamburger samples have been kept in refrigerator condition with 4 °C for 12 days and next, in days 0, 2, 4, 8 and 12 of preservtion they have been sampled and microbial tests (total count, *Salmonella, Staphylococcus*

aureus and mold and yeast) and sensory assessment (taste, odor and color) on three treatments have been repeated three times (anonymous, 1381).

Essential oil preparation and its analysis

Method of plant and garlic essential oil preparation

Garlic plant has been prepared form Barij Company in Hamedan province and next, the collected plants have been dried. The essential oil of the dried plant tissues has been extracted for three hours with the use of clevenger apparatus with the method of distillation with water and next their water of them has been extracted with the use of sodium sulfate without water and have been preserved in a closed containers with dark walls away from light and in refrigerator. Analysis of essential oils has been performed with the use of das chromatography device connected to a Mass spectrograph (GC/MS) (Thomas, 2004).

Garlic essential oil which is like a thick liquid has been diluted for preparing the desired concentration in distilled water, so that preparation of 0.03% concentration, 0.03 g of essential oil has been weighted and dissolved in 100 ml of distilled waterand for preparation of a 0.015% concentration, 0.015 g of essential oil has been dissolved in 100 ml of distilled water and then the prepared essential oil has been used in hamburger formulation.

Garlic essential oil is obtained with the use of distillation with steam with the use of clevenger apparatus of crushed garlic cloves. Analysis of the essential oil compounds has been performed by gas chromatograph device equipped withMass spectrometry (GC/MS).

Production and preparation of Nanoliposomes

Nanoliposomes are produced with the use of the method of direct injection of ethanol. Specified amounts ofphosphatidylcholine and cholesterol are dissolved in ethanol together with garlic essential oil. The obtained solution is injected with the use of a pump connected to a syringe in a specified amount of buffered saline solutionphosphate (PBS) and anti-freezing substance (Cryo-protectant) on Hot plate stirrer device. As soon as when ethanol solution is placed in contact with water, liposomes are formed.

Liposomes solution for final stabilization is stirred in room temperature for 15 minutes and in 50 °C temperature on stirrer device and finally as part of ethanol is separated with the use of evaporator system. A part of un-encapsulated essential oil after ultra-centrifuging Liposomes solutionin 60000 rpm is separated for 1 hour and then the rest of Liposomal suspension is lyophilized state with the use of Freeze drier device and is used in other stages of experiment. It should be mentioned that this part of the study will be conducted in Research Center for New Technologies in of Biological Sciences Engineering.

Preparation of cultivation media

Weighting: in this stage Utensils and scales with 0.1 g accuracy have been used.

Dissolving in distilled water: since distilled water doesn't contain Salts, cultivation media is dissolved quickly in it. For making liquid cultivation media after dissolving distilled water it has been poured into a pipe and it has been sterilized. However, for making solid cultivation media first boiling has been performed until achievement of sufficient clarity. final stage of Sterilization has been done with autoclave device (anonymous, 1386).

Microbial experiments

Total count of bacteria in hamburger

This test has been conducted according to national standard of Iran with number 5272 and the purplatemethod and plate count agar mediumhas been used. 30 ℃ of temperature and 72 hourse have been used.

Identification and count of Staphylococcus aureus

0.1 ml of prepared dilutions has been poured on plates containing cultivation media and have been expanded in the form of superficial cultivation in the media surface. After 24 hours it has been placed in a Greenhouse with 37 ℃. Black shiny convex colonies with diameters of 1.5 to 2.5 with a light hallo indicate the presence of Staphylococcus aureus that with counting and calculating the dilution the number of them has been calculated in each g (anonymous, 1384).

Salmonella test

For performing this test standard number 1810 has been used which includes three stage:

Pre-enrichment in non-selective liquid media

Sample incubation in buffered peptone water and keeping in greenhouse in 37 degrees for 16 - 20 hours.

Enrichment in selective liquid media

Incubation of the obtained cultivation from previous stage in RV (Rappaport Vassi) , MGP (Malachite Green Seya Pepton broth) , TTN (Tctrathionatenovbio cine broth) medias and keeping in greenhouse with 41.5 degrees for 24 hours.

Cultivation in solid media and identification

It is From the two obtained cultivation in the previous stage on two selective solid media (PRB(Phenol red brilliant green agar), SS(Salmonella shiglla), BS(Bismuth sulfite agar), XLD(xylose lysine deoxycholate agar)) and have been kept in 37 degrees for 24 - 48 hours in incubation (anonymos, 1381).

Mold and yeast counts

This test is conducted as per national standard of Iran with the number of 10899-1. The Rose Bengal broth in 25 greenhouse temperature for 3 to 5 days has been used. It should be mentioned that the used method is superficial cultivation method (anonymous, 1387).

Sensory tests

Sensory test includes color, taste, texture and general acceptance assessment with using a 5-point Hedonic method (with 5% error) by 8 of trained evaluators and completing questionnaires. 1 indicates the lowest and 5 indicate the highest score given by the evaluator (anonymous, 1386).

Statistical analysis

Data analysis. SPSS 16 has been used for statistical analysis. One-way variance test (ANOVA) has been used as well. For comparing the rotting course in various treatments in case of data normality repeated measure of ANOVA is used.

FINDINGS

Results related to the effect of garlic essential oils and nano-encapsulated garlic essential oil (with 0.033% and 0.015% concentrations) on total count of the bacteria of the preserved hamburger samples in refrigerated condition (4 degrees) for 12 days have been shown in figure 1.

Figure 1 . Comparison of logarithmic averages of total bacteria count of hamburger samples

Table 2 effect of nano-encapsulated garlic essential oil on total bacteria count in hamburger during preservation in refrigerator temperature for 12 days on the basis of (Log cfu/g) (Average ± standard deviation)

Figure 2. Comparison between average values of logarithmic total bacteria count of hamburger samples

The number of total bacteria count in control has significantly increased comparing to treatments with garlic essential oil with different concentrations during 12 days of preservation, in a way that its number in the end of the period has reached 9.50 Log cfu/g and also, total bacteria count in day 8 in the sample containing 0.015% concentration of garlic essential oil has reached 7.85 cfu/g, however, in concentration of 0.03% in day 8 the number of total bacteria count has reached 6.83 Log cfu/g and it has been changed at the end of the period to 7.90 Log cfu/g, which indicate to the positive effect of garlic essential oil concentration ($p<0.05$).

As it can be seen from figure 3-4, among the treated hamburger samples, the sample containing garlic essential oil with a concentration of 0.03% has less number of total bacteria count comparing to the control and sample containing 0.015% essential oil concentration (TI) over time. Therefore, we can say that increasing concentration and passage of time have a significant effect on bacteria number ($p<0.05$).

Total number of bacteria count in control sample significantly has increased comparing to treatments with nano-encapsulated garlic essential oil with different concentration during 12 days of preservation, in a way that in the end of the period it has reached 9.50 log cfu/g and also, the total number of bacteria count in day 8 in the sample containing 0.015% of nano-encapsulated garlic essential oil concentration has reached 7.14 log cfu/g, however, in 0.03% concentration in day 8 the number of total bacteria count has reached 6.77 log cfu/g and at the end of this period this number has changed to 7.27 log cfu/g, which indicate to a positive effect of nano-encapsulated garlic essential oil concentration ($p<0.05$).

As it can be seen in figure 4-4, among the treated samples of hamburger, the sample containing nano-encapsulated garlic essential oil with a concentration of 0.03% (T2) has less number of bacterial comparing to control sample and the sample containing nano-encapsulated garlic essential oil with 0.015% concentration (T1) with the passage of time, in a way that it can be said that increased concentration ahs a significant effect on the number of bacteria ($p<0.05$).

Result of *Staphylococcus aureus* count

Results related to the effect of garlic essential oil and nano-encapsulated garlic essential oil (in 0.03% and 0.015% concentration) on Staphylococcus aureus bacteria count of preserved hamburger samples in refrigerated condition (4 ℃).

Staphylococcus aureus count in control sample during preservation in 4 ℃ temperature for 12 days comparing to the treated sample with different concentrations of garlic essential oil indicates to an increasing course and has reached 4.74 log cfu/g at the end of the period and also in 0.015% concentration Staphylococcus aureus number has reach 3.65 log cfu/g in day 8 and at the end of the period has changed to 3.95 log cfu/g, however, in 0.03% concentration of garlic essential oil during the preservation period as we can see in table 4-1 in day 8 this number has reached 2.77 log cfu/g and at the end of the period it has reached 2.90 log cfu/g, which indicate to positive effect of garlic essential oil concentration on reducing the number of Staphylococcus aureus bacteria ($p<0.05$).

As it can be seen in figure 1, in highest concentration of garlic essential oil (0.03%) as the preservation time increases, the growth rate of bacteria reduces ($p<0.05$) and it can be said that the number of Staphylococcus aureus in treated samples with garlic essential oil has a significant difference with the same in control sample at 4 ℃ degrees temperature.

The number of Staphylococcus aureus in control sample during the preservation time in 4 ℃ degrees temperature for 12 days has an increasing course comparing to the treated samle with different concentrations of nano-encapsulated garlic essential oil and at the end of this period has reached 4.74 log cfu/g and also, in 0.015% concentration the number of Staphylococcus aureus in day 8 has reached 2.54 log cfu/g and at the end of the period has reached to 3.18 log cfu/g. however, in 0.03% of garlic essential oil concentration during the preservation time tables 4-2 shows in day 8 this number has reached 2.77 log cfu/g and at the end of the period it has reached 2.49 log cfu/g, that indicate to the positive effect of garlic essential oil concentration on reducing number of Staphylococcus aureus ($p<0.05$).

As figure 1 shows, in highest garlic essential oil concentration (0.03%), as the time of preservation increases, bacteria growth rate reduces ($p<0.05$) and it can be said that the number of Staphylococcus aureus in treated samples with nano-encapsulated garlic essential oil comparing to control sample in 4 ℃ temperature has a signfiiantdiffernce.

Results of Salmonella test

All tested hamburger samples have been negative in terms of the presence of Salmonella bacteria during the preservation days.

Results of Mold and yeast count

Results related to effect of garlic essential oil and nano-encapsulated garlic essential oil in 0.03% and 0.015% concentrations on the number of mold and yeast in preserved hamburger samples in refrigerator condition for 12 days has been shown in figure 3.

Table 4-6 effect of nano-encapsulated garlic essential oil on mold and yeast count in hamburger during preservation in refrigerator temperature for 12 days on the basis of (Log cfu/g) (Average ± standard deviation)

Figure 4-6 Comparison of the logarithmic average values of mold and yeast count in hamburger samples

The number of mold and yeast in control sample has increased significantly comparing to treated samples with garlic essential oil during 12 days of preservation, in a way that at the end of this period it has reached 5.36 Log cfu/g.

Also the produced hamburger sample with garlic essential oil with 0.03% concentration has lower number of Mold and yeast comparing to the sample with 0.015% concentration of the same with the passage of time, in a way that at day 8 the number of Mold and yeast has reached 3.44 Log cfu/g and at the end of the period it has reached 4.20 log cfu/g. in fact, with increasing the concentration the number of Mold and yeast has reduced. Statistical assessment indicate that garlic essential oil has a significant effect in inhibition of Mold and yeast growth in preserved hamburger samples in refrigerator temperature ($p<0.05$).

As figure 4-5 shows, with the passage of time the number of Mold and yeast assessed in the sample containing highest concentration of garlic essential oil (T2) has decreased comparing to control and T1 samples, which indicate to the positive effect of garlic essential oil concentration ($p<0.05$).

Results related to effect of nano-encapsulated garlic essential oil with 0.03% and 0.015% concentrations on Mold and yeast count of preserved hamburger samples in refrigerator condition for 12 days have been shown in tables 4-5 and 4-6.

The number of mold and yeast in control sample significantly has increased comparing to treated samples with garlic essential oil during 12 days of preservation, in a way that at the end of this period it has reached 8.36 log cfu/g.

Also, the produced hamburger with garlic essential oil of 0.03% concentration has less number of mold and yeast comparing to produced hamburger with garlic essential oil with 0.015% concentration with the passage of time, in a way that in day 8 this number has reached 3.44 log cfu/g and at the end of the period it has reached 4.80 log cfu/g. in fact, with increasing the concentration and passage of time the number of mold and yeast has reduced. Statistical evaluations indicate that garlic essential oil has a significant effect on inhibition of Mold and yeast growth in preserved hamburger samples in refrigerator temperature ($p<0.05$).

As figure 4-6 shows, with the passage of time the number of counted Mold and yeast in the sample with the highest concentration of garlic essential oil (T2) has decreased comparing to control and T1 sample, which indicates to positive effect of garlic essential oil concentration ($p<0.05$).

Results of cooking loss

No cooking loss has been observed in produced hamburger samples with garlic essential oil and nano-encapsulated garlic essential oil comparing to control sample and this can justify the application of essential oil in this industry.

Result of color assessment

No color changes has been observed in produced hamburger samples with garlic essential oil and nano-encapsulated garlic essential oil comparing to control sample and this as well can justify the use of this essential oil in this industry.

Results of sensory assessment

Results of sensory assessment of hamburger samples have been presented in table 1.

Table 1 - scores obtained from sensory assessment of hamburger samples

Figure 2 comparisons of obtained average values from sensory assessment of hamburger samples

Sensory assessment is shown in table 2-6.

Taste in terms of taste hamburger sample containing nano-encapsulated garlic essential oil with 0.03% concentration has a significant difference with other samples (P>0.05).

Odor in terms of odor there is no significant difference between the samples.

Color in terms of color also there is no significant difference between the samples.

Texture in terms of texture there is no significant difference between the samples.

As it is shown in figures 6 and 7, control sample does not have a significant difference with other samples in terms of color and texture, however, there is significant difference in terms of taste between the sample containing garlic essential oil with 0.03% concentration comparing with control sample and the sample obtaining 0.015% garlic essential oil at 0.05 level. However in terms of taste there is no significant difference between control sample and sample containing essential oil with 0.015% concentration (p>0.05).

Results of the present studies indicate that with adding normal garlic essential oil the odor and taste of the product is affected and the score allocated by testers has been reduced significantly. (p>0.05)

The highest reduction has been seen in 0.03% concentration of garlic essential oil, however, results obtained in this study indicate that with nano-encapsulation of garlic essential oil acceptance or the score given by testers in nano essential oil with 0.03% concentration does not have any significant difference with the form without any essential oil (p<0.05).

Statistical analysis indicates that hamburger samples containing garlic essential oil and nano-encapsulated garlic essential oil with 0.03% concentration has a significant difference in taste factor comparing to other samples (p<0.0001), however, no significant difference has been observed in terms of color, odor and texture.

DISCUSSION

Results of the present study indicate that hamburger samples containing 0.03% of garlic essential oil and nano-encapsulated garlic essential oil have less total number count of bacteria, Staphylococcus aureus, mold and yeast and also have the highest score of acceptance of testers specially in terms of taste that has shown as significant difference with the control sample.

Considering the obtained results from this study, in highest concentration of garlic essential oil and nano-encapsulated garlic essential oil (0.03%), as the duration of preservation increases, the growth rate of bacteria decreases and it can be said that the number of Staphylococcus aureus in treated samples with garlic essential oil and nano-encapsulated garlic essential oil has a significant difference with control samples in 4°C temperature. Consistent results have been obtained in a number of studies. For example, in a study 0.02%, 0.01% and 0.03% of garlic essential oil has been used in sausages formulation that the lowest count of Staphylococcus aureus at the end of preservation time has been found to be related to sample containing 0.03% of essential oil (Burt, 2004).

Pundor et al., (2010), have explored antimicrobial activity of garlic against Escherichia coli and a number of other bacteria and have found that these natural antibacterial compounds can be used as additives for increasing shelf time of food products.

Studies of Deresse et al. (2010) show the effect of antibacterial garlic extraction in inhibition of Staphylococcus aureusin laboratory environment. In this study, 30 different species of Staphylococcus aureus has been separated and the effect of Allium extracts Ethiopia has been studied in them. In this study it has been shown that MIC garlic water extraction should be more than 7.5 Milligrams per milliliterto be able to inhibit the growth of the bacteria. Also, it has been shown that if garlic water extraction is Autoclave, it will not be any more effective on any species of Staphylococcus aureus.

Aydin et al., (2007) have studies the antibacterial properties of chopped garlic on raw meat and hamburger. The results indicate that all treatments containing 10% of fresh garlic, both those that have been kept in room temperature and those that have been kept in refrigerator condition, after the passage of time have significantly reduced number of Aerobic mesophilic. However, in this study it has been shown that fresh chopped garlic both in room and refrigerator conditions have no significant effect on reducing growth of molds and yeasts.

Study of Gaysinsky et al. (2005) indicates that 0.15% concentration of encapsulated Eugenolin Sulfinol 485Q has the potential to inhibit theFour strains growth of Escherichia coli and three strains growth of Listeria monocytogenes.

Sallam et al. (2004) have studied the antibacterial effect of garlic on chicken salami and have found that adding garlic has a potential useful effect on preserving meat products. As per this study during a 21 days of preservation, 30 g fresh garlic or 9 g garlic power has a suitable effect in reducing APC (respectively, 6.42 and 6.94 CFU/g log10).

Spagno et al. (2013) has nano-encapsulated grape extract for using hazelnut sauce and have explored its effect in comparison with an extract that has not been encapsulated. Shelf life of the product with nano-encapsulated extract is 98 days, while in normal conditions it is 59 days.

Adding extract in the form of non-encapsulated reduces Peroxide value comparing to the product without extract; however, encapsulation of extract improves the efficiency of phenol compounds against lipid oxidation.

In the present study nano-encapsulated garlic essential oil has shown to have a desirable antimicrobial effect against existing bacteria in hamburger which has a significant effect on reducing microbe level and increasing shelf life of hamburgers ($p<0.05$). also, It has also shown that 0.03% concentration of garlic essential oil has the best antibacterial effect comparing to 0.015% concentration of the same and the taste of hamburger in 0.03% concentration of garlic essential oil is as well better and more desirable comparing to the sample with 0.015% concentration and control sample. Loss of cooking and change of color have not been seen in samples ($p>0.05$).

CONCLUSION

On the basis of obtained results it can be concluded that garlic essential oils and nano-capsulated garlic essential oils can be regarded as a natural preservatives in hamburger formulation for increasing shelf life of hamburger as well as improving its taste.

REFERENCES

1- Ayinechi, Y, Materia Medica and herbs of Iran. Publishing and Printing Institute of Tehran University. 1986: 324.
2- Amin, Gh, Traditional herbs Iran, Tehran. Research Center, Ministry of Health and Medical Education, 2001 (vol. 1): 40.
3- Burgner, P, 2001, Garlic Therapy: healing power of garlic, Nokhostin Publications; translated by Shohre Abdollahi.
4- Anonymous, national standard No. 2403, (2007), Institute of Standards and Industrial Research of Iran, Hamburger, characteristics and testing methods, 3rd edition.
5- Javidnia, K: Identification of existing compounds in plants essential oils of Shiraz, Ziziphora tenuior and chamomile species and their antimicrobial effects, using electrospray method- Mass spectrometry in studying nitro groups of compounds, PhD thesis of Pharmaceutical Chemistry, Tehran: University of Medical science, 1997.
6- Jawetz, A. (2004): medical microbiology of Jawetz. Translated by Bahador, A. (2004). Vol. 1, Samat Publications, pp 282 - 283, 287 - 290
7- Zargari, Ali, 1996. Medical plants, Publications University of Tehran.
8- Shahrani, M.M, Rafeean, H. A., Shirzad, M. Hashem Zadeh, H. Yousefi, and Khadivi, A.A, Amini, M. T., Moradi, J. Moghaddasi, M. Rahmani, M. Rahimi and D. Shahrani, Winter of 2006, Exploring the effect of Garlic methanol extract on acid secretion and pisin gastric in rats, research - scientific quarterly journal of Feiz, 10th period, issue 4, p. 8-12.
Anbarani, M. 1998, Food and medical characteristics of garlic and onion, Shahidi Pour Publication
9- Anbarani. M. 1998, Food and medical characteristics of garlic and onion, Shahidi pour Publications.
10- Ankri,D., Mirelman,D.,1999. Antimicrobial properties of allicin from garlic.Microbes and Infection.2.125-129.
11- Arora D. and Kaur J. (1999).Antimicrobial activity of spices. Int. J. Antimicrobial agents 2:257-262.
12- AydinA.K.BOsten.M.Emin and B.Bingol (2007) The Antimicrobial effect of chopped garlic in grund beef and raw meatball.jornal of medical food.203-207
13- Benkeblia,N.2004. Antimicrobial activity of essential oil extracts of various onions
(Allium cepa) and garlic (Allium sativum).Lebensm.-Wiss. u.-Technol. 37.263–268.
14- Bollman,J., Ismond,A., Blank,G.,2001. Survival of Escherichia coli O157:H7 in frozen foods: impact of the cold shock response. Int. J. Food Microbiol.64.127-138.
15- Carey,C., Kostrzynska,M., Thompson,S.,2009. Escherichia coli O157:H7 stress and virulence gene expression on Romaine lettuce using comparative real-time PCRJ.Microbiol.Metho.77.235-242.
16- Carey,C.M., Kostrzynska,M., Ojha.S., and Thompson.S.2008. The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic Escherichia coli O157:H7.J.Microbial.Method, 73:125-132.
17- Castellano,P., Belfiore,C., Vignolo,G.,2011. Combination of bioprotective cultures with EDTA to reduce Escherichia coli O157:H7 in frozen ground-beef patties. Food Control .22.1461-1649.
18- Chen, mu; wu .yy .(2002). Methods for preparation of meat and meat products for microbiological examination, meat hygiene.8:3-5.
19- Deresse D. (2010) Antibacterial effect of garlic (Aliumsativum) on staphylococcus aureus: an in vitro study. Asian journal of medical sciences 2(2) : 62-65
20- Donsi F, Annunziata M, Sessa M, Ferrari G: Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. LWT - Food Science and Technology. Volume 44, Issue 9, November 2011, Pages 1908-1914.
21- Doyle, M., Beuchat, L. and Montiville, T. (2001): Food microbiology: Fundamentals and Frontires, 2nded ed, ASM press: 193-208
22- Fathi M., Mozafari M.R., Mohebbi M., Nanoencapsulation of food ingredients using lipid based delivery systems, Trends in Food Science & Technology 23 (2012) 13-27.
23- Fujisawa H. ,k.Origuchi , H.Kumagai . (2008) Bialogical and chemical stability of garlic – derived Allicin, journal of agriculture and food chemistry,nihon university Graduate school Applied life science , Japan.
24- Breukink, E., de Kruijff, B, (1999), The lantibioticnisin, a special cade or not, Biochimica at biophysicaActa, 1467: 233-234.(23)
25- Harris,J., Cottrell,S., Plummer,S., Lloyd,D.2001. Antimicrobial properties of Allium sativum (garlic). Appl. Microbiol. Biotechnol.57.282–286.

26- Indu,M., Hatha,A., Abirosh,C., Harsha,U., Vivekanandan,G.2006. Antimicrobial activity of some of the south-indian spices against serotypes of Escherichia coli, salmonella, Listeria monocytogenes andAeromonashydrophila, Brazilian Journal of Microbiology, 37.153-158.

27- Kumar,M., Berwal,J.1998. Sensitivity of food pathogens to garlic (Allium sativum).Journal of Applied Microbiol. 84, 213–215.

28- Nedorostova,L., Kloucek,P., Kokoska,L., Stolcova,M., Pulkrabek,J.2009. Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. Food Control.20.157-160.

29- Nissen, H., Maugesten, T., Lea, P. 2001. Survival and growth of Escherichia coli O157:H7, Yersinia enterocolitica and Salmonella enteritidis on decontaminated and untreated meat. Meat Science, Vol 57, pp 291-298.

30- Ockerman H.W. and Y.M.Sun. (2000) Effect of different garlic products on chinese – style sausae. The Ohio state university department of animal sciences.

31- Parsaeimehr, M., Basti, A.A., Radmehr, B., Misaghi, A., Abbasifar, A., Karim, G., Rokni, N. et al. 2010. Effect of ZatariamultifloraBoiss. essential oil, nisin, and their combination on the production of enterotoxin C and α-Hemolysin by Staphylococcusaureus. Foodborn pathogens and diseas. 7(3):299-305.

32- Pundir,R., Jain,P., Sharma,C.2010. Antimicrobial Activity of Ethanolic Extracts of Syzygiumaromaticum and Allium sativum Against Food Associated Bacteria and Fungi.Ethnobotanical Leaflets. 14: 344-60.

33- Razavi Rohani,M., Moradi,M., Mehdizadeh,T., Saei-Dehkordi,S.,2011. The effect of nisin and garlic (Allium sativum L.) essential oil separately and in combination on the growth of Listeriamonocytogenes.LWT-Food Scien.Technol.44.2260-2265.

34- Sakagami, Y.; Kaioh, S.; Kajimura, K. and Yokoyamma, H. (2000), Inhibitory effect of clove extract on vero-toxin production by enterohemorrhagicEscherichiacoli O157:H7. Biocontrol Science, 5, 47-49.

35- Sallam,K., Ishioroshi,M., Samejima,K.2004. Antioxidant and antimicrobial effects of garlic in chicken sausage.Lebensm.-Wiss. u.-Technol. 37.849–855.

36- Souza,E., Stamford,T., Lima,E., Trajano,V., Filho,J.2005. Antimicrobial Effectiveness of Spices: an Approach for Use in Food Conservation Systems. Braz. Arch. Biol. Technol.48.549-558.

37- Yin,M., Cheng.W.2003. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Sci. 63 .23–28.

38- Zhong Z, Yu X, Zhu J. Red bayberry extract inhibits growth and virulence gene expression of the hum

39- Seydim, A.C. and G. Sarikus, Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Research International, 2006. 39: p. 639-644.

40- Pranoto, Y., S.K. Rakshit, and V.M. Salokhe, Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT, 2005. 38: p. 859-865.

41- Benkeblia, N., Antimicrobial activityof essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). LWT, 2004. 37: p. 263-268.

42- Yin, M.-c. and W.-s. Cheng, Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat science, 2003. 63: p. 23-28.

43- Aguirrezabal, M.M., et al., The e□ect of paprika, garlic and salt on rancidity in dry sausages. Meat science, 2000. 54: p. 77-81.

44- Mariutti, L.R.B., et al., Effect of sage and garlic on lipid oxidation in high-pressure processed chicken meat. European Food Research and Technology, 2008. 227: p. 337-344.

45- Pranoto, Y., V.M. Salokhe, and S.K. Rakshit, Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. Food Research International, 2005. 38: p. 267-272.

46- Jirukkakul, N., A study of Mu Yor sausage wraps using chitosan films incorporating garlic oil, lemon grass oil and galangal oil. International Food Research Journal, 2013. 20(3): p. 1199-1204

48- Spigno, G., et al., Nanoencapsulation systems to improve solubility and antioxidant efficiency of a grape marc extract into hazelnut paste. Journal of Food Engineering, 2013. 114: p. 207-214.

49- Wu, Y., Y. Luo, and Q. Wang, Antioxidant and antimicrobial properties of essential oils encapsulated in zein nanoparticles prepared by liquideliquid dispersion method. LWT- Food Science and Technology, 2012. 48: p. 283-290.

50- Gaysinsky, S., et al., Growth inhibition of Escherichia coli O151: H 1 and Listeria monocytogenes by carvacrol and eugenol encapsulated in surfactant micelles. Journal of Food Protection, 2005. 65(12): p. 2552-2566.

51- Olfati A, Moghaddam GH, Moradi Kor N, Bakhtiari M. 2014. The Relationship between Progesterone and Biochemical Constituents of Amniotic Fluid with Placenta Traits in Iranian Crossbred Ewes (Arkhar-Merino×Ghezel). Asian Pacific Journal of Tropical Medicine. 7 (Suppl 1): S162-S166.

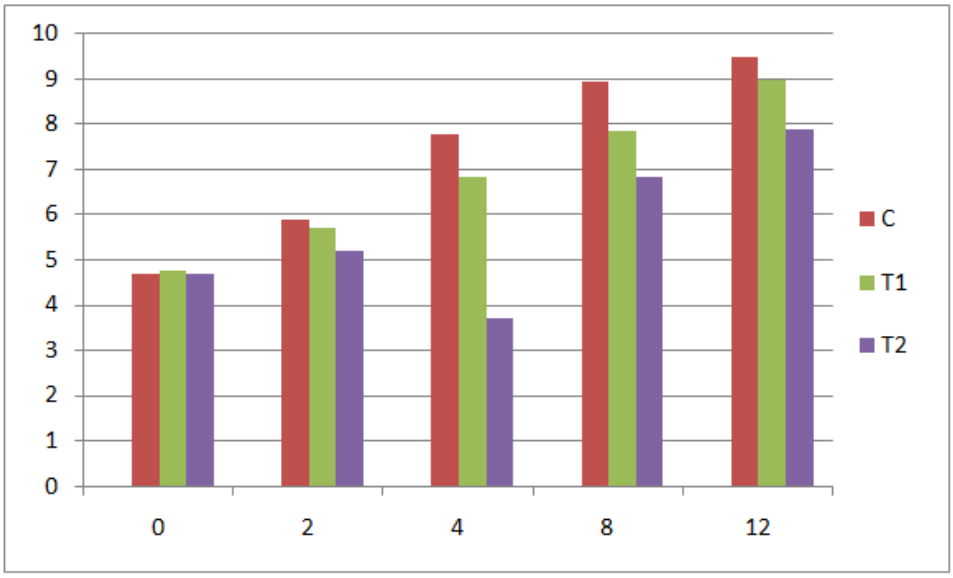

Figure 1: Comparison of logarithmic averages of total bacteria count of hamburger samples

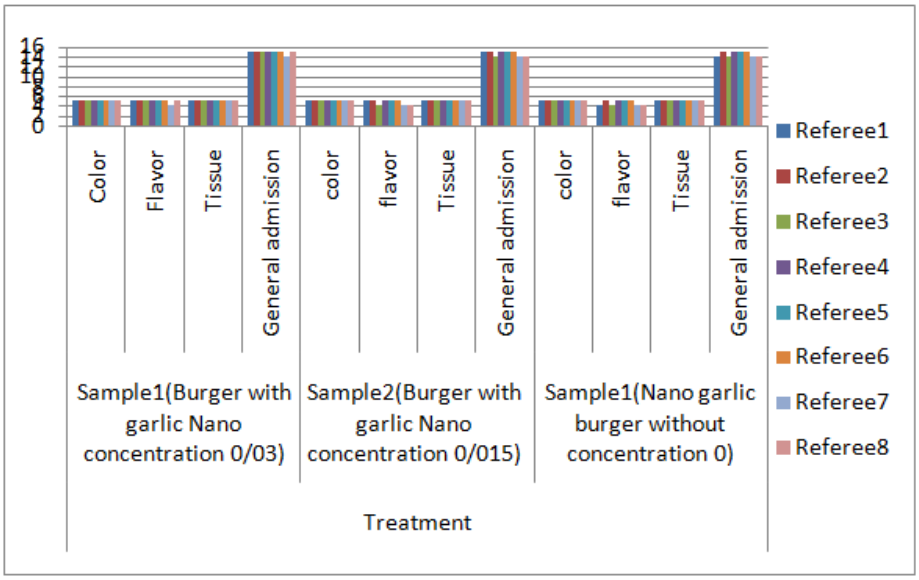

Figure 2: comparison of obtained average values from sensory assessment of hamburger samples

Table 1: The effect of garlic essential oil on total bacteria count in preserved hamburgers in refrigerated temperature for 12 days as per (logcfu/g) (average ± standard deviation)

Control/treatments	0	2	4	8	12
Produced hamburger without garlic essential oil (control)	4/70±0/01^{a}	5/90±0/01^{b}	7/80±0/01^{d}	8/96±0/01^{f}	9/50±0/01^{g}
Produced hamburger with garlic essential oil 0.015%	4/75±0/01^{a}	5/71±0/01^{b}	6/83+0/01^{e}	7/85±0/01^{d}	8/98±0/01^{f}
Produced hamburger with garlic essential oil 0.03%	4/69±0/01^{a}	5/20±0/02^{c}	6/72±0/01^{e}	6/83±0/01^{e}	7/90+0/01^{d}

Table 2: Effect of nano-encapsulated garlic essential oil on total bacteria count in hamburger during preservation in refrigerator temperature for 12 days on the basis of (Log cfu/g) (Average ± standard deviation)

Control/treatment	0	2	4	8	12
Produced hamburger sample without nano-encapsulated garlic essential oil (control)	4/70±0/01^{a}	5/90±0/01^{b}	7/80±0/01^{d}	8/96±0/01^{e}	9/50±0/01^{h}
Produced hamburger sample with nano-encapsulated garlic essential oil (0.015%)	4/62±0/01^{a}	5/30±0/02^{c}	5/38±0/01^{c}	7/14±0/03^{f}	7/68±0/01^{d}
Produced hamburger sample with nano-encapsulated garlic essential oil (0.03%)	4/63±0/01^{a}	4/98±0/01^{a}	5/63±0/01^{c}	6/77±0/01^{g}	7/27±0/02^{d}

(C: control, T1: treatment with 0.015% concentration of garlic essential oil, T2: treatment with 0.03% concentration of garlic essential oil)

Table 3: effect of garlic essential oil on Staphylococcus aureus count in hamburger during preservation in refrigerated temperature for 12 days on the basis of log cfu/g (average ± standard deviation)

Control/treatments	0	2	4	8	12
Produced hamburger without garlic essential oil (control)	2/3±0/03^{a}	2/47±0/01^{a}	3/17±0/03^{c}	3/92±0/01^{e}	4/74+0/01^{g}
Produced hamburger without garlic essential oil 0.015% concentration	2^{b}	2/30±0/02^{a}	2/60±0/01^{ad}	3/65±0/01^{e}	3/95+0/01^{eh}
Produced hamburger without garlic essential oil 0.03% concentration	2^{b}	2/20±0/03^{a}	2/54±0/01^{ad}	2/77±0/01^{df}	2/90±0/01^{fi}

Average number of Staphylococcus aureus (log cfu/g) Number of preserved days in refrigerator

Table 4: effect of nano-encapsulated garlic essential oil on Staphylococcus aureus count in hamburger during preservation in refrigerator temperature for 12 days on the basis of (Log cfu/g) (average ± standard deviation)

Control/treated	0	2	4	8	12
Produced hamburger without nano-encapsulated garlic essential oil	2/3±0/03[a]	2/47±0/01[a]	3/17±0/03[c]	3/92±0/01[d]	4/74±0/01[d]
Produced hamburger with nano-encapsulated garlic essential oil with 0.015% concentration	2[b]	2/20±0/05[a]	2/39±0/01[a]	2/54±0/01[a]	3/18±0/01[c]
Produced hamburger with nano-encapsulated garlic essential oil with 0.03% concentration	2[b]	2/23±0/02[a]	2±0/05[a]	2/77±0/01[a]	3/49±0/01[c]

(C: Control, T1: treated with 0.015% concentration of nano-encapsulated garlic essential oil, T2: treated with 0.03% concentration of nano-encapsulated garlic essential oil

Table 5: effect of garlic essential oil on Mold and yeast count on hamburger during preservation in refrigerator temperature for 12 days on the basis of (log cfu/g) (Average ± standard deviation)

Control/treated	0	2	4	8	12
Produced hamburger without garlic essential oil (Control)	<2[a]	2/25±0/02[a]	4/49±0/01[b]	5/62±0/01	5/36±0/02[e]
Produced hamburger with garlic essential oil with 0.015% concentration	<2[a]	2/23±0/02[a]	3/55±0/01[c]	3/93±0/01	4/50±0/01[b]
Produced hamburger with garlic essential oil with 0.03% concentration	<2[a]	2±0/05[a]	2/54±0/01[a]	3/44±0/01[c]	4/20±0/01[b]

Average number of Mold and yeast (log cfu/g) Preserved days in refrigerator

(C: control, T1: treated with 0.015% garlic essential oil concentration, T2: treated with 0.03% garlic essential oil concentration)

Table 6: Average values of sensory assessment of hamburger samples during 12 days of preservation

Control/treatments	Color	Taste	Texture	Total acceptance
Control	5	51**0**/52± /4	5	17**0**/83± /4
Hamburger with garlic essential oil 0.015% concentration	5	51**0**/38±/3	5	17**0**/46± /4
Hamburger with garlic essential oil 0.03% concentration	5	51**0**/38±/4	5	17**0**/46±/4

sensory assessment parameters

Table 7: Average values of sensory assessment of hamburger samples during 12 days of preservation

Control/treatments	Color	Taste	Texture	Total acceptance
control	5	510/37± /3	5	170/45± /4
Hamburger nano-encapsulated garlic essential oil with 0.015% concentration	5	510/37±/3	5	170/45± /4
Hamburger nano-encapsulated garlic essential oil with 0.03% concentration	5	530/50±/4	5	170/83±/4

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Study of Flora, Life Form and Chorotypes of the Rykan Area of West Azerbaijan (Iran)

Leila Mirzaei[1]* and Leila Joudi[2]

[1]Young Researcher and Elites club, Islamic Azad University, Urmia Branch, Urmia, Iran.
[2]Department of Agriculture, Shabestar branch, Islamic Azad University, Tabriz, Iran.

Received: 22 Mar 2015 Revised: 24 Apr 2015 Accepted: 29 May 2015

***Address for correspondence**
Leila Mirzaei
Young Researcher and Elites club,
Islamic Azad University, Urmia Branch,
Urmia, Iran
Mobile: +989144391957
E-mail: l.mirzaei_2009@yahoo.com

ABSTRACT

Floristic study of each region is really significant and it indicates the existence of plants and their condition. Therefore, studying geographical origin and floristic Study of each region are considered to be the most effective methods for management and protecting existing reservations. The Rykan area is 8 km from the city of Urmia city in Iran.In this study, 273 samples were collected and dried plant and then transferred to the herbarium and the national identification keys universal and the scientific name. In total, 190 species belonging to 128 genera and 43 families were identified for this zone. Among them, 157taxa are dicots and 33 are monocots. Family Compositae with 27 species and Poaceae with 23 species are the largest families. The life form of plant species was determined by using of Raunkier's method and they are including hemi cryptophytes (55%), followed by Therophytes (32%), Phanerophytes (7%), Chamephytes (4%) and Geophytes(2%). Also chorological investigation on the species of this area showed about56 % of the species belongs to Irano - Turanian region.

Key words: systematic-herbarium -flora- Rykan-taxonomy.

INTRODUCTION

Iran with approximately 1.65 million square kilometer surface area in terms of topography, vegetation, climate and geographical features is a larg country and except for Turkey it is the richest country in the Middle East in terms of

plant diversity (White et al, 1991; Attar et al, 2014). According to a recent study, flora of Iran includes 8000 species (22% endemic species) belonging to 1450 genera and 150 families. These families include 124 dicotyledons, 22 monocoty-ledons and 4 gymnosperms (Ghahreman,1994;Akhani, 2006; Mozaffarian, 2007). The rich flora of the country is the consequence of locating among three main phytochoria in the Old World including Irano- Turanian, Euro-Siberian and Sahara-Sindian, (Zohary, 1973).The life form of any plant is fixedto development based on morphological adaptation of plants to environmental conditions. Also depends on genetics and environmental factors. According to plant communities in different climates can be of different form and there are different classification of the life form but in Raunkier system is most commonly used that is based on the position of vegetative buds observed after a unfavorable for growth season. Plants are divided in the six main groups: Phanerophyte, Cryptophyte, Chamaephyte, Hemicryptophyte, Therophyte and Epiphyte (Asri, 1999). The spectrum of dominant life forms represents how adaptation of all plants Recognize is introduction for every research. Study plant biodiversity is important for optimum using of plant species in the different fields such as Ecology, Agriculture, Medicine and many other aims that make life on earth possible and enjoyable and most effective methods in the management and protection existence reservoirs (IranNezhad et al,2001; Akbarinia et al, 2004; Ejtehadi et al, 2005; Kerstin et al, 2013).Generaly ,to evaluate the status of biodiversity and to determine how current conservation efforts can be improved, biodiversity monitoring is crucial.Our study had been carried out for the first time in Northwest Iran with the aim to precisely identify plant speciesand life forms.

MATERIALS AND METHODS

The our study region located in geographical limitation between (52°58’28”) and (52°42’15”) easternlongitude and (28°35’20”) and (28°20’15”) northern latitude in north western of Iran. First of all the basic information in this study is gathered and then some visit from the region is done. The collected plant taxa were dried according to herbarium techniques, and numbered then recorded.The Flora of Iranica (Rechinger, 1998-2005), Flora Orientalis (Boissier ,1936), Flora of Iraq (Townsen et al, 1985), Flora of Turkey (Davis, 1965– 1985), Flora of Iran (Asadi et al, 1988-2011), Colored Flora of Iran (Ghahreman,1977–2007)and other applicable resources and references were used for the identification of the specimens. Plant samples were identified at the Islamic Azad University herbarium in Urmia. The Diagram of abundance was designed by EXCEL and the frequency of families,and specieswas calculated(Figure1).The life forms of plants were determined by Raunkier method and related diagrams were drawn (Raunkier, 1934). The life form of plants is an adaptive response to environment and provides an ecological classification that may be indicative of habitat conditions (Archibold, 1995). Geographical distribution of species was determined based on vegetative areas classified by Zohary (Zohary, 1963-1973) and Takhtajan (Thakhtajan, 1986).The life-form spectrum of the plants investigated is presented in fig 2.

RESULTS

In this study a total number of 190 species has been identified belong to 128 genera and 43 families. In appendix of the paper a checklist of all species collected in area is shown with information about their life forms and chorological types (Table 1). Plant species for our study are belonging to Angiosperm expectone genera Equisetum. Among identified families of Angiospermae, 32 families are monocotelydole and other belong to dicotyledonous (157).The most important and biggest families are Asteraceae (27species), Poaceae (23 species), Fabaceae (17 species), Brassicaceae (17 species) and Lamiaceae (11 species).

It is concluded from the results of the study that the study area is very rich with reference to plant diversity .In this research, we obtain in stydu thepercentages of life forms hemicryptophytes (55%), therophytes (22%), phanerophytes (7%), chamaephytes (4%), Geophytes 2% of the flora of the area.

According to Arcihold (1995) the frequency of Hemicryptophyte plants is due to cold and to altitude climate. Hemicryptophytes can be survived by their buds that they laied below and near soil surface or in the dried rosette leaves at soil surface. The high proportion of therophytes in this area show the arid condition of summer and the cold winter that often adapted to shortage rainfall, spend vegetative period in the form of seed (Asri, 2003; Asaadi, 2009; Nadaf et al, 2011; Ejtehadi et al, 2003; Mobayen, 1980-1996; Memariani et al, 2009).Therophytes complete their life cycle during favorable season and survive in the form of seed(Shahsavari,1998).The low percentage of Phanerophyte, Chamaephyte and Geophytes shows that they are not adapted to climate and edaphically situations area. Among all plants Hemicryptophyte is dominant and the rophyte with is in the next order. In fact life forms of the plants indicate the possibility of adaptation of plants to environmental factors especially climatic condition. Therefore, the geographical distribution of plant species reflects the climate conditions and adaption of plants to area (Asri, 2003; Rahimi et al, 2013).The geographical distribution of plants Considering to this fact that56% plant species in this area are Irano-Turanian elements, we can conclude that this area belongs to Irano- Turanian (Ghahreman et al, 2006;Khodadadi et al, 2009; Mataji et al, 2013; Heydari et al, 2013).

DISCUSSION

On the whole, the frequencies of the rophyte and Hemicryptophyte among the plants of the area show the effects of the two types of climate: Mediterranean and cold temperate. Hemicryptophyte adapted to conditions of the area by using different ways such as: reserving water, using ground water, reducing water needs by losing leaves and diminishing own vegetative growth (Najafi,2005).The rophyte adapted to the rainfall shortage and dryness of the region, by enduring in the form of seed during the vegetation season. The dominance of Hemicryptophyte and The rophyte clearly indicates adaptation of these plants to area aridity. The geographical distribution of plants reflects the climate conditions(Abbasi,2012) Considering the fact that 56% plant species in the area are Irano-Turanian elements, there can be concluded that the area is Irano-Turanian (characterized by low rainfall and extended dry season in worlds).

CONCLUSION

The study area is very rich in terms of plant diversity. Documenting habitat floristic composition is valuable for ecological research continuation as well as management and conservation of plants and animals. Resources available for conservation of species and ecosystems are in short supply relative to the needs. Targeting conservation and management actions toward the species and ecosystems requires clearly established priorities such as studies of floristic composition. Thus, in this research, the identification of 190 plant species in Rykan region with their chorology, family, species and life form are of central importance for further ecological investigation, conservation and management of wildlife refuge in Iran.

ACKNOWLEDGMENTS

Authors are thankful to Young Researcher and Elite club (Islamic Azad university Urmia branch) for providing financial assistance in the form of a major research project. Special thanks to Dr.Ashkan Khoda bandehlou, for helping me with my experiments.

REFERENCES

1.Abbasi S, Afsharzadeh S & Mohajeri A. 2012. Study of flora, life forms and chorotypes of plant elements in postural region of Yahya Abad (Natanz). J. Plant. Bio, 4(11): 1-12.

2- Akbarinia M, Zare H, Hosseini SM and Ejtehadi H. 2004. Study on vegetation structure, floristic composition and chorology of silver forest of Hyrcanian region. J. Pagouhesh and Sazandegi 64: 84-96.
3- Akhani H .2006.Flora Iranica: facts and figures and a list of publications by K. H. Rechinger on Iran and adjacent areas. Rostaniha 7(suppl. 2):19–61.
4- Archibold O W. 1995. Ecology of world vegetation. London, Chapman and Hall Inc.
5- Assadi M .1988-2011. Flora of Iran. Vol: 1-71 Research Institute of Forests and Rangelands press,Tehran.
6- Asaadi AM. 2009. Floristic study of Firozeh watershed in Khorasan province. Research journal of Biological Sciences 4 (10): 1092-1103.
7- Asri Y, 1999. Ecological study of arid zone plant (Case study: biosphere reservoir, province), PhD dissertation, Islamic Azad University Science and Res.
8- Asri Y, 1998. Vegetation of salt marsh in Uromia Semirom (Isfahan Province), Iran. Pajouhesh-valake plains, Forests and rangelands Research Sazandegi. 18(3): 84-103.
9- Asri Y, 2003. Plant diversity in the biosphere Rangeland Publications. reservoir Desert, Forest and rangelands Research Institute Publication, 326: 305.
10- Attar Roshan S, Heydari M. Short Communication: Flora, Life Form And Chorological Study Of Quercus Brantii Habitat In Emamzadeh Abdullah Woodland, Iran. BIO DIVERSIT Volume 15. 2014 Pages: 245-250.
11- Davis PH. (ed.) .1965 – 1985.Flora of Turkey. Edinburgh University Press, Edinburgh.
12- Ejtehadi H, Amini T, Zare H. 2005. Importance of Vegetation Studies in Conservation of Wildlife: A Case Study in Miankaleh Wildlife Refuge, Mazandaran Province, Iran. Environ. Sciences. 9: 53-58.
13- Ghahreman A, 1994. Iran chromophytes (systematic plant) Volume 4, Tehran University Publication Center.
14- Ghahreman A, Heydari J, Attar F and Hamzeh'ee B. 2006. A floristic study of southwestern slopes of Binalood elevations (Iran: Khorassan province). Journal of Science (University of Tehran) (JUST), 32(1): 1-12.
15- Ghahreman A (1977-2007). Colored flora of Iran.Vols 1-26, Research Institute of Forests & Rangelands press,Tehran.
16- Heydari M, Poorbabaei H, Hatami K, Salehi A, Begim Faghir M. 2013. Floristic study of Dalab woodlands, north-east of Ilam province, west Iran. Iranian J Sci Technol 37: 301-308.
17- Iran Nezhad Parizi M H, Sanei Shariat Panahi M, Zobairy M and Marvi Mohajer M. 2001. Floristic and vegetation of the Park National Geographic Khabar and Rouchun wildlife protection, J. Iran. Natural Reso, 54(2): 111-127.
18- Khodadadi S, Saedi mehrvarz SH, Naqinezhad A. 2009. Contribution to flora, and habitats of the Estil wetland (Astara) and its surroundings, Nourth west Iran, Rostaniha 10(1) 44-62.
19- Mataji A, Kia Daliri H, Babaie S, Jafari S, Attar Roshan, S. 2013. Flora diversity in burned forest areas in Dehdez, Iran. Folia forestalia polonica, Series A, 55 (1): 33-41.
20- Memariani F. and Joharchi M R.2007. Taxonomic revision and notes on species diversity and phytogeography of the genus Allium L. (Alliaceae) inNE Iran. Abstract book of the 1st National Plant Taxonomy Conference of Iran. 6th of September,Tehran, pp. 3-4.
21- Meymandi Nezhad M J , 1973. Zagros vegetation flora of Kobar watershed in Ghum area, Iran. disturbance. J. Mohitshenasi, 1: 97-107.
22- Mobayen S. 1985. Vegetation of Iran. Volume 3, Tehran University Press.
23- Mozafarian V. 2007. A Dictionary of Iranian Plant Names. Farhang Moaser Pub.
24- Nadaf M, Mortazavi M, Halimi Khalilabad M. 2011. Flora, life forms and Chorotypes of plants of Salok protected area in Khorasan province. Pakistan journal of Biological Sciences 14 (1): 34-40.
25- Najafi Tireh-Shabankareh K, Jalili A , Khorasani N, Jamzad Z & Asri Y. 2005. Flora, life forms and chorotypes of plants in the Genu protected area, Hormozgan province (Iran). Pajouhesh. Sazandegi,18(4): 50-62.
26- Rahimi A and Atri M. 2013. Study of flora of Miandasht Wildlife Refuge in Northern Khorassan Province, Iran (a), Journal of Ecology and the Natural Environment Vol. 5(9), 241-253.
27- Rechinger KH. 1963-2009. Flora Iranica, Akademische Drucku Verlagsanstalt,Graz.
28- Raunkier C. 1934. Life forms of plants. Oxford University Press, Oxford.
29- Shahsavari A, 1998. Natural forest and Iran .Forests and Ranglands Research Institute (Iran). 111: 79.
30- Thakhtajan A. 1986. Floristicegions of the world. University of California Press Ltd.

31- Townsend C.C and Guest E. 1966 – 1985. Flora of Iraq. Minstry of Agriculture and Agrarian Reform, Baghdad, Iraq.

32- White F. and Leonard J. 1991. Phytogeographical links between Africa and Southwest Asia. Flora Veg. Mundi, 9: 229-246.

33- Zohary M. 1963. On the geobotanical structure of Iran.Bulletin of the Research Council of Israel, Section D Botany, Supplement 11D, 1–114.

34- Zohary M. 1973. Geobotanical Foundations of the Middle East. 2 vols. Gustav Fischer Verlag.

35- Olfati A, Moghaddam GH, Moradi Kor N, Bakhtiari M. 2014. The Relationship between Progesterone and Biochemical Constituents of Amniotic Fluid with Placenta Traits in Iranian Crossbred Ewes (Arkhar-Merino×Ghezel). Asian Pacific Journal of Tropical Medicine. 7 (Suppl 1): S162-S166.

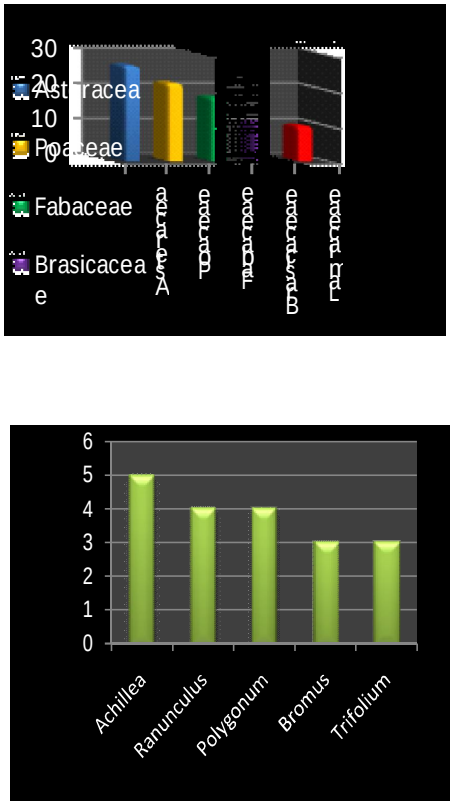

Fig.1. Ferequency of tha family and species of plants in study area

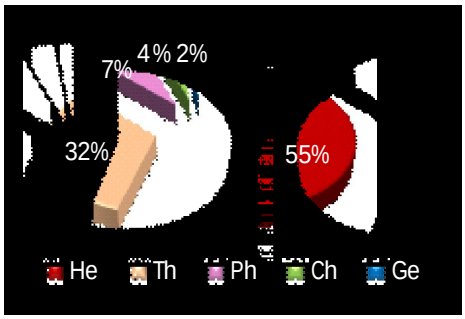

Fig.2. Life-form spectrum of plants in study area

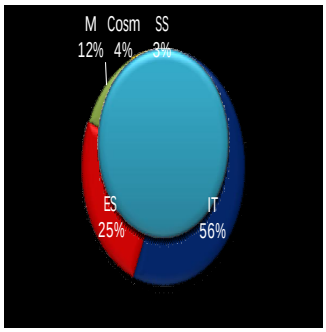

Fig.3. Chorological types spectrum in flora in study area.

Chorotype	Life form	Species	Genus	family	No
IT-M	Th	A.aestivalis L.	Adonis	Rununculaceae	1
IT-ES	Th	C.falcatus (L.) Pers.	Ceratocephalus	Rununculacea	2
IT	Th	R.lomatocarpus D.C.	Ranunculus	Rununculacea	3
IT	He	R.polyanthemos L.	Ranunculus	Rununculacea	4
IT-ES	Th	P. glaucum Boiss.	Papaver	Papaveraceae	5
Cosm	Th	F.vaillantii Loisel.	Fumaria	Fumariaceae	6
Cosm	Th	C.album.L	Chenopodium	Chenopodiaceae	7
Cosm	Th	A.chlorostachyswilld.	Amaranthus	Amaranthaceae	8
IT-ES-M	Th	V.pyramidata Medic.	Vaccaria	caryophylaceae	9
IT-ES-SS	Th	P.convolvolus L.	Polygonum	Polygonaceae	10
IT	Th	Polygonum avicular L.	Polygonum	Polygonaceae	11
IT	He	R.chalepensis Miller.	Rumex	Polygonaceae	12
IT	He	R.conglomeratus Murr.	Rumex	polygonaceae	13
Cosm	He	R.crispus L.	Rumex	polygonaceae	14
IT-ES-M	Th	M. negelecta Wallr.	Malva	Malvaceae	15
IT	He	C.spinosa L.	Capparis	Capparidaceae	16
IT	He	A.bracteatum Boiss. & Buhse.	Alyssum	Brassicaceae	17
Cosm	Th	C.bursa-pastoris (L.)Medik.	Capsella	Brassicaceae	18
IT-ES-M	Th	D.sophia (L.) Schur.	Descurainia	Brassicaceae	19
IT	He	E. cuspidatum DC.	Erysimum	Brassicaceae	20
IT	Th	L.latifulium L	Lepidium	Brassicaceae	21
IT-ES-M	Th	N.apiculata C.A.M.	Neslia	Brassicaceae	22
IT	Th	S. irio L.	Sisybrium	Brassicaceae	23
IT	He	R. luteola L.	Reseda	Resedaceae	24
Cosm	He	M .sativa L.	Medicago	Fabaceae	25
IT-ES	He	M .albus Medicus	Melilotus	Fabaceae	26
IT	Ch	S.pachycarpa C.A.Mey.	Sophora	Fabaceae	27
IT-ES-M	He	T.repens L	Trifolium	Fabaceae	28
IT-ES-M	Th	T. arvense L.	Trifolium	Fabaceae	29
IT	Th	T. spruneriana Boiss.	Trigonella	Fabaceae	30
IT	He	E.boissieriana (Woron) Prokh.	Euphorbia	Euphorbiacea	31
IT	He	Z.eichwaldii C.A.M.	Zygophyllum	Zygophyllaceae	32
IT-ES-M	Th	E.ciconium(L.) L Her.	Erodium	Geraniaceae	33
IT	He	D.carota L.	Daucus	Apiaceae	34
IT	He	F.vulgaris Bernh.	Falcaria	Apiaceae	35

IT-ES	Ph	L.ruthenicum Murry	Lycium	Solanaceae	36
IT	He	C.betonicifoliussp.pedunc ularis(Boiss)Parrismiller.s ub.	Convolvulus	Convolvulaceae	37
IT-M	Th	A.bracteosa Boiss.	Alkanna	Boraginaceae	38
IT-ES-M	He	A. italica Retz.	Anchusa	Boraginaceae	39
IT-ES	Th	A. procumbens L.	Asperugo	Boraginaceae	40
IT-M-SS	Th	L. amplexicaule L.	Lamium	Lamiaceae	41
IT-ES	He	M.longifolia L.	Mentha	Lamiaceae	42
IT	He	S.nemorosa L.	Salvia	Lamiaceae	43
IT-ES	He	S.persica	Stachys	Lamiaceae	44
IT-M-SS	He	P. lacceolata L.	Plantago	Plantaginaceae	45
IT-E	He	p. major L.	Plantago	Plantaginaceae	46
IT-M	Th	V.anagallis-aquatic L.	Veronica	Scrophulariaceae	47
IT-ES	Th	G.humifusum Bieb	Galium	Rubiaceae	48
IT	Th	A.micrantha willd.	Achillea	Asteraceae	49
I	He	A.tenuifolia lam.	Achillea	Asteraceae	50
IT	He	A.vermicullaris Trin.	Achillea	Asteraceae	51
IT-M	Th	C.pycnocephalus L.	Cardus	Asteraceae	52
IT	He	C.abrotanoides L.	Carpesium	Asteraceae	53
IT-M	Th	C.oxyacantha M.B	Carthamus	Asteraceae	54
IT	He	C. virgata Lam.	Centaurea	Asteraceae	55
IT	He	C. cyanus L.	Centaurea	Asteraceae	56
IT	He	C.triumfetti All.	Centaurea	Asteraceae	57
IT	Th	C.benedictus L.	Cnicus	Asteraceae	58
IT-ES	Ch	C.intybus L.	Cichorium	Asteraceae	59
IT	He	C.calcitrapa Boiss.	Cousinia	Asteraceae	60
IT	Th	C.sancta(L.)Babcock.	Crepis	Asteraceae	61
IT-M-ES	He	L.serriola L	Lactuca	Asteraceae	62
IT	He	S.mollis willd.	Senecio	Asteraceae	63
IT-M-ES	Th	S.vulgaris L.	Senecio	Asteraceae	64
IT-M	He	S.tenerrimus L.	Sonchus	Asteraceae	65
IT -ES	He	T.syriacum Boiss.	Taraxacum	Asteraceae	66
IT	Ge	T.marginatus Boiss. & Buhse.	Tragopogon	Asteraceae	67
IT	Ge	T. pratensis L.	Tragopogon	Asteraceae	68
IT	Th	A.triuncialis L.	Aegilops	Poaceae	69
IT	Th	A.trichophorum (Link)Richter.	Agropyron	Poaceae	70
IT-M	Th	A.wiestii steud.	Avena	Poaceae	71
Cosm	Ge	B.beneckenii (Lang)Trimen.	Bromus	Poaceae	72

ES	Th	B.tectorum L.	Bromus	Poaceae	73
IT	He	C. dactylon (L.) Pres.	Cynodon	Poaceae	74
IT-ES-M	He	D.glomerata L.	Dactylis	Poaceae	75
IT-ES-M	Th	E.crus gall.	Echinochloa	Poaceae	76
IT	Th	E.confusum melderis.	Eremopypum	Poaceae	77
IT	Th	E.distans K. Koch .	Eremopypum	Poaceae	78
IT-M	Th	H.glaucum steud.	Hordeum	Poaceae	79
IT-ES-M-SS	Th	L.persicm L.	Lolium	Poaceae	80
IT-ES	Th	P. paniculatum Hudson. var paniculatum	phleum	Poaceae	81
IT-ES	He	P.phleoides (L.) H. Karst.	Phleum	Poaceae	82
IT-SS	He	P.bulbosa L.	Poa	Poaceae	83
IT-ES	Th	S.glauca (L) P.Beauv.	Setaria	Poaceae	84
IT-ES	He	A.rotundum	Allium	Liliaceae	85
IT-ES	He	A.rubellum M. Bieb.	Allium	Liliaceae	86
IT	Ge	M. neglectum Guss..	Muscari	Liliaceae	87
IT-ES	He	M.longipes Boiss.	Muscari	liliaceae	88
IT-ES	He	I.tataricum (Pall.) Herb.	Ixilirion	Amaryllidaceae	89
ES-SS	He	C.divulsa stokes	Carex	Cyperaceae	90
IT-ES	ph	J.regia L	Juglans	Juglandaceae	91
IT-ES	He	C.acutum L	Cynancum	Asclepiadaceae	92
IT	He	E. hirsutum L.	Epilobium	Onagraceae	93
IT-ES	Ph	U. minor Miller	Ulmus	Ulmaceae	94
IT	Ph	V. vinifera L	Vitis	Vitaceae	95
IT-ES	Ch	S .schlschlegleevii Sosn.	Stachys	Lamiaceae	96
IT-ES	He	C. sosnovskyi Grossh.	Centaurea	Asteraceae	97
Cosm	He	C. sylvatica Griseb.	Calystegia	Convolvulaceae	98
IT- ES	He	Z. Fabago L.	Zygophylum	Zygophyllaceae	99
IT- ES	Th	E. sativa Lam	Eruca	Brasicaceae	100
Cosm	Th	C. Draba (L.) Desv	Cardaria	Brasicaceae	101
IT	He	R.tuberosus L.	Rumex	Polygonaceae	102
IT	Th	C. orientalis (Gay) Schrod	Consolida	Ranunculaceae	103
IT	He	R. luteola L	Reseda	Resedaceae	104
IT-ES-M	He	M. officinalis (L.) Desr	Melilotus	Fabaceae	105
IT	Ch	A. Astragalusmicrocephalus Willd.	Astragalus	Fabaceae	106
IT	Ph	S. alba L	Salix	Salicaceae	107
IT	Th	D. dasystachyumBoiss&.Bal.	Delphinium	Ranunculaceae	108
IT-ES	Cr	. G. verum L	Galium	Rubiaceae	109
IT	He	S. alopecuroides L	Sophora	fabaceae	110
IT-ES	Cr	. E. arvense L	Equisetum	Equisetaceae	111

IT	He	L. officinale L.	Lithospermum	Boraginaceae	112
IT	Ge	R. tinctorum L.	Rubia	Rubiaceae	113
IT	Ch	P. recta L	Potentilla	Rosaceae	114
IT	He	A. officinalis L.	Althaea	Malvaceae	115
IT-ES	Ph	S. Dulcamara L.	Solanum	Solanaceae	116
IT-ES	He	I. reticulata M. B.	Iris	Iridaceae	117
IT-ES	Th	G. persicum. SchonbeckTemesy	Geranium	Geraniaceae	118
IT	He	V.szovitsianum Boiss.	Verbascum	Scrophulariaceae	119
IT- ES	He	V. cracca L.	Vicia	Fabaceae	120
Cosm	Th	S. nigrum L	Solanum	Solanaceae	121
IT	He	E. virgata Walds.	Ephorbia	Ephorbiaceae	122
IT	He	C. varia L.	Coronilla	fabaceae	123
IT- ES	He	U. dioica L.	Urtica	Urticaceae	124
IT	He	S.bupleuroides L.	Silene	Caryophylaceae	125
M-IT- ES	Th	S. irio L.	Sisymbrium	Brassicaceae	126
M-IT- ES	Th	arvense L. .T	Trifolium	Fabaceae	127
IT	Th	X. longipapposum Fisch.	Xeranthemum	Asteraceae	128
ES	Cr	J. inflexus L.	Juncus	Juncaceae	129
IT-ES	Ph	R.canina L.	Rosa	Rosaceae	130
IT-M	Th	.V .peregrine L	Vicia	fabaceae	131
IT	Th	S.ongiflora Boiss.Hausskn	Saturejea	Lamiaceae	132
IT-ES-M	He	G. glabra L.	Glycyrrhiza	Fabaceae	133
IT	He	.N. persica Boiss	Nonnea	Boraginaceae	134
IT	Th	P. lapathifolium L.	Polygonum	Polygonaceae	135
IT	Ph	E. angustifolia L	Eleaegnum	Elaeaegnaceae	136
IT-ES-M	He	T. chamadris L.	Teucrium	Lamiaceae	137
Cosm	Th	D. stramonium L.	Datura	Solanaceae	138
IT	Ph	A. carduchorum Bornm.	Amygdalus	Rosaceae	139
IT	Ph	A. spinosa L.	Atraphaxis	Polygonaceae	140
IT	Ph	Pyrus. sp	Pyrus	Rosaceae	141
IT	He	L.P. harmala	Peganum	Zygophylaceae	142
IT-M	Ch	R. damascens Miller	Rosa	Rosaceae	143
IT-ES-M	Ph	F. carica L.	Ficus	Moraceae	144
IT	He	A . talyschensis A. Fedor	Anthemis	Asteraceae	145
IT	He	A. aucheri Boiss.	Alceae	Malvacea	146
IT-ES-M	He	P. europaean L.	Plumbago	Plumbaginaceae	147
IT	He	BenthS. Montbor Auch. ex.	Salvia	Lamiaceae	148
IT	He	L. corniculatus L.	Lotus	Fabaceae	149
IT-ES	Ph	A. fominii Kusn	Acantholimon	Caryophyllaceae	150
IT-ES	Cr	R. oxyspermus Willd.	Ranunculus	Ranunculaceae	151
IT-ES	He	C. spinosa L.	Capparis	Capparidaceae	152

IT-ES	He	A. millefolium L	Achillea	Asteraceae	153
ES	Cr	M. parviflorum Fisch.	Marrubium	Lamiaceae	154
IT- ES	Th	Hyoscyamus niger L.	Hyoscyam	Solanaceae	155
IT-ES	He	N. Officinale (L.) R.Br.	Nasturtium	Brassicaceae	156
IT-ES-M	He	A.orientalis (L.) Drude.	Astrodacus	Apiaceae	157
IT-ES-M	He	M. neglecta Wallr.	Malva	Malvaceae	158
IT-M	Th	P. sativum L.	Pisum	Fabaceae	159
IT	He	A. bibersteinii Afan.	Achillea	Asteraceae	160
IT	He	A. orientalis	Alkanna	Boraginaceae	161
IT	He	A. arundinaceus Poir.	Alopecurus	Poaceae	162
IT	He	A. basineri Trautv.	Astragalus	Fabaceae	163
IT	He	E. boissieriana(Woron) Prokh	Euphorbia	Euphorbiaceae	164
IT	Ge	M. neglectum Guss.	Muscari	liliaceae	165
IT	Ch	O. melanotricha Boiss.	Onobrychis	Fabaceae	166
IT	Th	P. argemone L.	Papaver	Papaveraceae	167
IT	Th	S. iberica M.B.	Scandix	Apiaceae	168
IT-ES-M	He	V.S.T.azerbaijanicum	Taraxacum	Asteracea	169
IT	Th	R.arvensis L.	Ranunculaceae	Ranunculaceae	170
Cosm	He	P. lasiothrix Fedde.	Papaver	Papaveraceae	171
IT-M	Th	Ch.botrys L.	Chenopodium	Chenopodiaceae	172
IT	Th	R. refracta DC.	Roemeria	Papaveraceae	173
IT	He	S. chlorifolia Sm.	Silene	Caryophylaceae	174
IT	He	S. orientalis (Boiss) Sojak.	Scariola	Asteracea	175
IT	He	S. azerbaijan Grau.	Scrophularia	Scrophulariaceae	176
IT-M-ES	Th	G. tuberosum L.	Geranium	Geraniaceae	177
IT-ES	He	E. pungens Trautv.	Echinops	Asteracea	178
IT-ES	He	C. lanatus L.	Carthamus	Asteracea	179
IT-M-ES-SS	Cr	A. repens LP Beauv.	Agrropyron	Poaceae	180
IT	Th	A. sterilis L.	Avena	Poaceae	181
IT-M-ES-SS	Cr	M. persica Kunth.	Melica	Poaceae	182
IT-ES	He	P. repens L.	Panicum	Poaceae	183
IT	Th	B. danthoniae Trin.	Bromus	Poaceae	184
IT	Th	P. annua L.	Poa	Poaceae	185
IT	Cr	G. reticulate (Pall.) Schult.	Gagea	Liliaceae	186
IT- ES	Cr	S. diziensis Grossh.	Scilla	Liliaceae	187
IT	Ph	C. incana(pall.) Spach.	Cerasus	Rosaceae	188
IT- ES	Ph	S. crenata L.	Spirea	Rosaceae	189
IT- ES	C	T. kotschyanus Boiss.	Thymus	lamiaceae	190

Tabel. 1. Plant families, species, life forms and chorotypes in Rykan area

Ph:phanerophyt,Th:Therophyte,He:Hemicryptophyte,Ch:Champhyt,Cr:Cryptophyt,IT:Irano-Touranear,M:Mediteranear,ES:Europe Siberean, Cosm:Cosmoplitan.

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Energy Use Trends in Horticultural Crop Production at Various Mechanization Levels (Case Study: Buin Zahra)

Houshang Ordikhani[1]*, Iraj Ranjbar[1], Mohammad Ghahderijani[2] and Mohammad Gholami Parashkoohi[1]

[1]Takestan Branch, Islamic Azad University, Takestan, Iran
[2]Science and Research Branch, Islamic Azad University, Tehran, Iran.

Received: 19 Mar 2015 Revised: 21 Apr 2015 Accepted: 29 May 2015

***Address for correspondence**
Houshang Ordikhani
Takestan Branch, Islamic Azad University,
Takestan, Iran.
E-mail: hooshang_or@yahoo.com

ABSTRACT

Energy use pattern in the production of four horticultural crops (pistachio, nectarine, peach, and apple), which are the main horticultural crops of Buin Zahra, were studied. The dependent variables of this research were energy efficiency (ratio), energy output to energy input ratio, energy productivity, and net energy gain. Preliminary questionnaires were designed to collect the required information, and were evaluated as the initial pretest in interviews with a number of fruit growers in the region. The independent variables of the research were the planting method (mechanized and conventional), land area used for fruit production, and orchard ownership. In the studied region, the maximum amount of energy (6300 MJ/ha obtained from fossil fuels) was used in peach production, and 33% of the total energy input was obtained from fossil fuels: fossil fuels provided the largest share of the total energy used, with chemical fertilizers and manure ranking as the second and third energy providers, respectively. The largest amount of energy obtained from chemical fertilizers (5800 MJ/ha) was in peach production. Energy ratios in the production of the same crops were different in orchards with small and large land areas, with large orchards having higher energy ratios compared to small ones. The research offered strategies for upgrading energy productivity in fruit production in the studied region.

Key words: Energy, orchard, peach, nectarine, apple, pistachio, Buin Zahra

INTRODUCTION

Agriculture is an energy conversion process in which solar energy, energy obtained from fossil fuels, and electrical energy are converted into foodstuff and fibers required by man. In primitive agriculture, which relied on human power and on the sun, what was harvested was a little more than what was planted (Helsel, 1993). More investments were needed to provide food and satisfy the other needs of the ever-increasing human populations, so that with the passage of the centuries animal power was employed, followed a little later by water and wind power (which replaced animal power). With these changes, humans had more free time and energy, and more and cheaper energy at their disposal compared to the past (Koochaki and Hosseini, 1994). Horticulture is a subsector of agriculture and 33% of the added value (1990 prices), 47.8% of agricultural exports, and 78.4% of export values in the agriculture sector belonged to the horticulture subsector in 2002 (Deputy Ministry of Horticulture in the Ministry of Agriculture Jihad, 2002). Energy is classified as direct energy and indirect energy. Direct energy is used to produce the inputs such as chemical fertilizers, pesticides, machinery, etc. If the energy used for production is available and can be converted to another form in the short term, it is called renewable; and it is called non-renewable energy if this conversion cannot take place (Ozkan et al, 2003).

In this research, the effects of the area of land used for fruit production and the mechanization pattern employed in producing fruit crops (peach, nectarine, pistachio, and apple) in Buin Zahra were studied. The purposes of the research were:

1. To study the current situation regarding costs of fruit crop production (peach, nectarine, pistachio, and apple) in the region

2. To determine the share of input with respect to energy used per unit of fruit crop production (peach, nectarine, pistachio, and apple)

3. To compare and evaluate energy efficiency and productivity in fruit crop production (peach, nectarine, pistachio, and apple).

In research conducted in West Azarbaijan Province on evaluation of energy efficiency in apple orchards, the calculated output energy was 6500000 kilocalories and the input energy 8669447.6 kilocalories: energy efficiency, or energy ratio, was 0.75.

Chomsing et al. (2006) studied energy requirements of five crops including rice, corn, sugar cane, and soybean in three regions of central, northern, and southeastern Thailand and found sugar cane required 8.81 GJ/ha. Merini (2001) conducted a study on sugar cane energy requirement in small and large fields in Morocco and found it to be 47.83 and 64.9 GJ/ha, respectively.

MATERIALS AND METHODS

In this section, the study area (Buin Zahra), the methodology, the dependent and independent variables, the statistical population and sampling method, the measurement tool, the main methods of collecting information, and the statistical methods used for analyzing the data are described.

The dependent variables of the study were energy efficiency (ratio), profit to cost ratio (the ratio of energy output to energy input), energy productivity, and net energy gain. The independent variables included the land area of the orchards, agricultural systems, and experience and education levels of fruit growers. To acquire the required

information, preliminary questionnaires were designed and used as pre-test in interviews a number of fruit growers in the region. The independent variables of the research were the various levels of mechanization (mechanized and conventional), land area of the orchards, and orchard ownership. Based on this, a number of villages in Buin Zahra were first randomly selected. Names of fruit growers in the selected villages were listed and, using random sampling and employing proportional allocation, the questionnaires were distributed among them. The Cochran formula was used to find the sample volume. Cochran introduced the following formula for calculating the number of required samples (n) (Mansoorfar, 1997)

$$n = Nt^2s^2/Nd^2 + t2s^2$$

In the above relation, N is the statistical population (the number of fruit growers), and t the acceptable confidence coefficient obtained from the t-student table (assuming normal distribution of the related feature). Moreover, S^2 is the variance of the studied feature in the population (variance of energy efficiency in the study region in this research), d the desired probability accuracy (half the confidence interval), and n the sample volume. Using this formula, sample volume was obtained. Energy use indicators: The standard energy factors and indicators of energy ratio, net energy gain (NEG), energy productivity, and energy intensity (specific energy) are required to study energy use in the region and compare it with those in other regions.

RESULTS AND DISCUSSION

Table 4 lists the energy balance for the different fruit crops in the study region and also the economic indicators and the energy indices.

As shown in table 2, energy ratio in large orchards is, in some cases, different from and, in other cases, larger compared to small orchards. This means that energy use efficiency in the production of some horticultural crops improved with increases in the land area of the orchards. Pistachio orchards with 2.08 had the maximum energy ratio, while this ratio was less than one for peach orchards. However, energy ratio did not vary with the size of nectarine orchards (it was about 0.94 for small and large orchards).

The effects of land area of the orchards and agricultural systems on energy efficiency of studied horticultural crops

This section studied the effects of agricultural systems on energy efficiency in horticultural crop (peach, nectarine, apple, and pistachio) production. This factor was divided into four classes. Table 4-3 shows results of ANOVA. As shown in this table, the mutual effects of orchard size (smaller than 0.5 and larger than 0.5 hectare) and agricultural systems on energy efficiency were not significant, but the effects of orchard size and agricultural systems on energy efficiency were significant at the 5 and 1% levels, respectively.

Clearly, fruit growers that live solely on income received from their orchards pay closer attention to fruit production. Moreover, they spend more time on their orchards. Duncan's test was used for comparing the means to further study various agricultural systems and the reasons why their effects were significant.

CONCLUSION AND SUGGESTIONS

Energy ratios in large orchards were different from those in small ones (and were higher in some cases). This means that energy use efficiency in the production of some horticultural crops improved with increases in land area used in fruit production. Pistachio with 2.01 had the maximum energy ratio, but this ratio was less than 1 in nectarine orchards (it was about 0.95 in both small and large orchards).

As this research indicated, the effects of both orchard size (larger or smaller than 0.5 hectare) and agricultural systems on energy efficiency were significant: the effects of the land area used for fruit production and of agricultural systems on energy efficiency were significant at the 5 and 1% levels, respectively.

REFERENCES

1. Helsel ZR. Energy and alternatives for fertiliser and pesticide use. In: Fluck RC, editor. Energy in world agriculture, 6. Elsevier Science Publishing; 1992. p. 177–210.
2. Darlington , D., 1997. What is efficient agriculture? Available at URL: http:// www.veganorganic .net/agri.htm
3. Pimentel, D. Energy inputs in production agriculture.1999. In: R.C. Fluck (Ed), Energy in Farm Production, Elsevier, Amsterdam, pp. 13 – 29.
4. Ozkan, B., H. Akcaoz and F. Karadcniz, 2003. Energy requiremcnt and economic analysis of citrus production in Turkey, Energy Conversion and Management , Vol. 44.46-56
5. 53- Ozkan B, Akcaoz H, Fert C. Energy input output analysis in Turkish agriculture. Renewable Energy .2004;29(1):39–51.
6. Kitani,O. CIGR, Handbook of agricultural engineering volume 5, Energy & Biomass Engineering.1998. ASAE publication
7. Biermann, s., G. W. Rathke, K. J. Hulsbergen & W.Diepenbrock, 1999. Energy recovery by crops in dependence on the input of mineral fertilizer . Martin – Lutther King University.
8. Canakci, M., M. Topakci, I. Akinci and A. Ozmerzi, 2005. Energy use partern of some field crops and vegetable production : Case study for Antalya region , Turkey . Energy Conversion and Management , Vol. 46.pp 655-666

Figure 1: The geographical location of Buin Zahra in Ghazvin Province

Table 1: Energy input and output and energy indices of horticultural crop production at various levels

			Peach	Nectarine	Apple	Pistachio
Orchards smaller than 0.5 hectares	Energy input	Fuels energy	6300.00	3600.00	5100.00	3458.00
		Energy expenditure of orchard workers	670.00	580.00	980.00	685.00
		Machinery depreciation energy	1260.00	720.00	1020.00	691.60
		Chemical fertilizers energy	5800.00	5360.00	4800.00	4638.00
		Pesticides energy	1340.00	1400.00	1890.00	2500.00
		Water energy	576.00	685.80	558.00	549.00
		Electrical energy	3200.00	3810.00	3100.00	3050.00
		Total	19146.00	16155.80	17448.00	15571.60

	Energy output	Crop yield	40000.00	28000.00	40000.00	1400.00
		Crop energy	18000.00	12600.00	40000.00	28000.00
		Crop residue energy	3960.00	2772.00	8800.00	6160.00
		Total	21960.00	15372.00	48800.00	34160.00
	Energy indicators	Energy ratio	1.15	0.95	2.80	2.19
		Energy productivity	2.09	1.73	2.29	0.09
		Net energy gain	2814.00	-783.80	31352.00	18588.40
	Economic indicator	Profit to cost ratio	1.67	1.27	1.83	2.55
Orchards larger than 0.5 hectare	Energy input	Fuels energy	8800.00	8400.00	7450.00	4300.00
		Energy expenditure of orchard workers	335.00	290.00	490.00	342.50
		Machinery depreciation energy	2816.00	2688.00	2384.00	1376.00
		Chemical fertilizer energy	12300.00	11560.00	11680.00	8600.00
		Pesticide energy	4600.00	4200.00	3560.00	5600.00
		Water energy	1036.80	1234.44	1004.40	988.20
		Electrical energy	5760.00	6858.00	5580.00	5490.00
		Total	35647.80	35230.44	32148.40	26696.70
	Energy output	Crop yield	48000.00	32000.00	46000.00	2200.00
		Crop energy	40800.00	27200.00	46000.00	44000.00
		Crop residue energy	8976.00	5984.00	10120.00	9680.00
		Total	49776.00	33184.00	56120.00	53680.00
	Energy index	Energy ratio	1.40	0.94	1.75	2.01
		Energy productivity	1.35	0.91	1.43	0.08
		Net energy gain	14128.20	-2046.44	23971.60	26983.30
	Economic indicator	Profit to cost ratio	2.08	1.63	2.15	2.93

Table 2: Average input and output energies in various orchards together with economic indicators and energy indices

Crop type		Peach	Nectarine	Apple	Pistachio
Energy input	Fuels energy	7550.00	6000.00	6275.00	3879.00
	Energy expenditure of orchard workers	502.50	435.00	735.00	513.75
	Machinery depreciation energy	2038.00	1704.00	1702.00	1033.80
	Fertilizer energy	9050.00	8460.00	8240.00	6619.00
	Pesticide energy	2970.00	2800.00	2725.00	4050.00
	Water energy	806.40	960.12	781.20	768.60
	Electrical energy	4480.00	5334.00	4340.00	4270.00
	Total	27396.90	25693.12	24798.20	21134.15
Energy output	Crop yield	44000.00	30000.00	43000.00	1800.00
	Crop energy	29400.00	19900.00	43000.00	36000.00
	Crop residue energy	6468.00	4378.00	9460.00	7920.00
	Total	35868.00	24278.00	52460.00	43920.00

Energy indices	Energy ratio	1.31	0.94	2.12	2.08
	Energy productivity	1.61	1.17	1.73	0.09
	Net energy gain	8471.10	-1415.12	27661.80	22785.85
Economic indicator	Profit to cost ratio	1.88	1.46	2.00	2.76

Table 3: ANOVA of energy efficiency at four farm sizes and four agricultural systems in wheat production

Sources of variation	Degree of freedom	Sum of squares	Mean squares	F
Area under cultivation	1	14.96	14.96	70.06*
Agricultural system	3	4.76	1.59	22.32**
Farm size * Agricultural system	3	0.70	0.10	1.42 ns
Replication	129	7.56	0.05	0.82
Error	257	18.29	0.071	
Total	303	754.49		

The symbols ns, *, and ** stand for not significant and significant at the 5 and 1% levels, respectively.

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Case Report of Concurrent Peritoneopericardial Diaphragmatic Hernia and Hypertrophic Cardiomyopathy in a Persian Cat

Seyed Milad Vahedi[1]*, Mohammad Reza Esmailinejad[2], Seyed Farzin Seyednejad[1], Hamed Mansoor Lakooraj[3], Seyed Mohammad Mojtahedzade[1], Donya Aghaee Nezhad[1], Paria Samadi Tari[1] and Daryoush Shirani[1]

[1]Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Iran.
[2]Department of Radiology and Surgery, Faculty of Veterinary Medicine, University of Tehran, Iran.
[3]Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Iran.

Received: 18 Mar 2015 Revised: 19 Apr 2015 Accepted: 28 May 2015

***Address for correspondence**
S. M.Vahedi DVM
Department of Internal Medicine,
Faculty of Veterinary Medicine,
University of Tehran,Tehran, Iran-14155-6453
E-mail: miald_vahedi@ut.ac.ir, Mobile: +98-9124116288

ABSTRACT

A Peritoneopericardial diaphragmatic hernia is a defect which permits direct communication between the peritoneal and pericardial cavities through a defect in the diaphragm. An eight-year-old Persian cat was referred with a history of anorexia, lethargy, and weight loss. Increased pulse rate, heart rate and respiratory rate were considerable. Abnormal heart sound and a systolic murmur were detected on auscultation of left side of the thorax. In radiographic study the cardiac silhouette was inclined to the thoracic wall in consequence of the pressure from caudal aspect. In echocardiography, hypertrophic cardiomyopathy was diagnosed and liver border found attached to the heart. Dilation of both atrium was detectable in both long and short axes and thickening of the muscular layer of the left ventricle was noticeable. In ultrasonography examination a 137 mm defect was found in the diaphragm and followed by some part the liver parenchyma, gall bladder was herniated to the chest cavity. A conclusive diagnosis of peritoneopericardial diaphragmatic hernia was made by data collected from physical examination, radiography, echocardiography, and ultrasonography study. Hernia repair surgery was recommended after initial treatment of hypertrophic cardiomyopathy. PPDH is diagnosed with difficulty and is rarely differentiated from other diaphragmatic hernias. This is the first report that describes the PPDH concurrent with hypertrophic cardiomyopathy in a Persian cat.

Key words: peritoneopericardial diaphragmatic hernia, hepertrophic cardiomyopathy, Persian cat.

INTRODUCTION

Peritoneopericardial Diaphragmatic Hernia in a Persian Cat

Peritoneopericardial diaphragmatic hernia (PPDH) is a common incidental finding in cats and is rarely symptomatic [8]. The disease is the most common congenital defect involving the pericardium in dogs and cats [11]. In PPDH, abdominal organs are herniated into the pericardial sac. There is no direct communication between the thoracic and peritoneal cavities in normal dogs and cats [1]. This communication can occur as a result of congenital or acquired hernias. The defect that results in a PPDH caused by abnormal development of the septum transversum [3 and 4]. It seems that this abnormality is not heritable [4 and 7]. PPDH in humans can be acquired secondary to trauma; whereas, in cats and dogs, it is almost congenital [3, 5 and 9]. Dogs with PPDH can live asymptomatic for years [3, 4 and 7]. In cats PPDH can be associated with polycystic kidney disease, portosystemic shunts, hepatic cysts, myelolipomas, and chyelothorax associated with lung lobe torsion [11]. Cardiac abnormalities can occur with PPDH. This anomaly may occur concomitantly with other congenital defects such as hydrocephalus, umbilical hernias, abnormal swirling of hair on the ventral abdomen, intra-cardiac defects, and pulmonary vascular [2]. This report describes the clinical and radiological features of peritoneopericardial hernia in a Persian cat.

Case description

An eight-year-old Persian cat with a history of anorexia, lethargy, tachypnea, and weight loss was referred to Small Animal Teaching Hospital, Faculty of Veterinary Medicine, University of Tehran. Increased pulse rate, heart rate and respiratory rate were considerable. Abnormal heart sound and a systolic murmur were detected on auscultation of left side of the thorax. Laboratory examination revealed mild neutropenia and elevated alkaline phosphatase level. In radiographic study Vertebral Heart Score was 10 but liver was smaller than normal and cardiac silhouette was inclined to the thoracic wall in consequence of the pressure from caudal aspect. Cranially displacement of gastric axis was also seen in lateral view. Increased opacity of lung lobes on both sides and air bronchogram was also seen (Fig. 1). In echocardiography, hypertrophic cardiomyopathy (HCM) was diagnosed and liver border found attached to the heart. Dilation of both atrium was detectable in both long and short axes and thickening of the muscular layer of the left ventricle was noticeable (Fig. 2,3 and 4). In ultrasonography examination a 137 mm defect was found in the diaphragm and followed by some part the liver parenchyma, gall bladder was herniated to the chest cavity (Fig. 5). Treatment of HCM was done with furosemide and captopril. Hernia repair surgery was recommended after initial treatment.

DISCUSSION

The purpose of this report is to discuss the clinical features and diagnosis of PPDH in a Persian cat. In this case the main complaint of client was related to the respiratory system (dyspnea, cough) and digestive tract disorder (anorexia, weight loss) which are the most clinical signs of PPDH. These signs mostly demonstrated within the first year of life but in some cases are identified after years [2, 6 and 10]. Although Abdominal discomfort or swelling, shock and collapse have been reported [10]. Physical examination findings in animals with PPDH are often ascites, muffled heart sounds, murmurs caused by displacement of the heart and ventral abdominal wall defect. The most commonly organ herniated to chest is the liver [3 and 4]. In this case abnormal heart sound and a murmur was detected in physical examination and hypertrophic cardiomyopathy and liver herniation was revealed in echocardiography and radiography investigation. However, it is impossible to determine which event occurred earlier. Although surgical repair of the hernia is the recommended treatment [10], priority was given to the management of HCM before any surgical correction. This report is the first report of PPDH in a cat in Iran and is the

first report of this defect associated with HCM. In summary, this report demonstrates the fact that PPDH may be subclinical until a complicating factor like hypertrophic cardiomyopathy occurs.

REFERENCES

1. Liptak, J.M., Bissett, S.A., Allan, G.S., Zaki, S., Malik, R. (2002). Hepatic cysts incarcerated in a peritoneopericardial diaphragmatic hernia. J. Feline Med. Surg., 4(2): 1235.
2. Statz, G.D., Moore, K.E., Murtaugh, R.J. (2007). Surgical repair of a peritoneopericardial diaphragmatic hernia in a pregnant dog. J. Vet. Emerg. Crit. Care, 17(1): 77–85.
3. Berry, C.R., Koblik, P.D., Ticer, J.W. (1990). Dorsal peritoneopericardial mesothelial remnant as an aid to the diagnosis of feline congenital peritoneopericardial diaphragmatic hernia. Vet. Radiol., 31(5): 239-245.
4. Fossum, T.W. (1997). Surgery of the lower respiratory system: pleural cavity and diaphragm, In: Fossum TW. (Eds.). Small Animal Surgery. St. Louis, USA: Mosby, p. 685–687.
5. Fossum, T.W. (2000). Pleural and extrapleural diseases, In: Ettinger SJ, Feldman ED. (Eds.). Textbook of Veterinary Internal Medicine. Philadelphia, USA: WB Saunders, p. 1098–1111.
6. Levine, S.H. (1987). Diaphragmatic hernia. Vet. Clin. North Am. Small Anim. Pract., 17:411–430.
7. Johnson, K.A. (1993). Diaphragmatic, pericardial, and hiatal hernias, In: Slatter D. (Eds.). Textbook of Small Animal Surgery. Philadelphia, USA: WB Saunders, p. 455–469.
8. Noden, D.M., deLahunta, A. (1985). Malformations of the diaphragm, In: Noden DM, deLahunta A. (Eds.). The Embryology of Domestic Animals. Developmental Mechanisms and Malformations. Baltimore, USA: Williams and Wilkins, pp. 290.
9. Evans, S.M., Biery, D.N. (1980). Congenital peritoneopericardial diaphragmatic hernia in the dog and cat: A literature review and 17 additional case histories. Vet. Radiol., 21(3): 108-116.
10. Reed, J.R. (1988). Pericardial diseases. In: Miller MS, Tilley LP, Fox RP (Eds.). Canine and feline cardiology. New York, USA: Churchill Livingstone, pp. 495.
11. Kheirandish, R., Saberi, M., Vosough, D., Askari, N. (2014). Congenital peritoneopericardial diaphragmatic hernia in a terrier dog. Vet. Res. Forum, 5(2): 153 – 155.

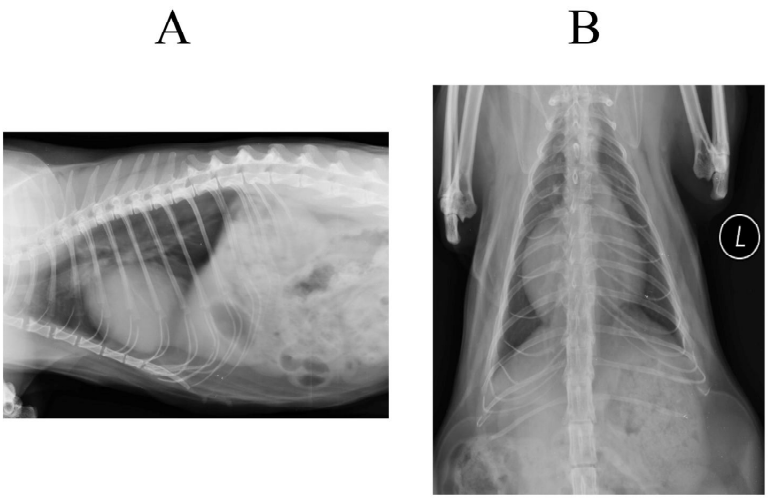

Fig 1. Right lateral (A) and dorsoventral (B) views of the thoracic cavity.

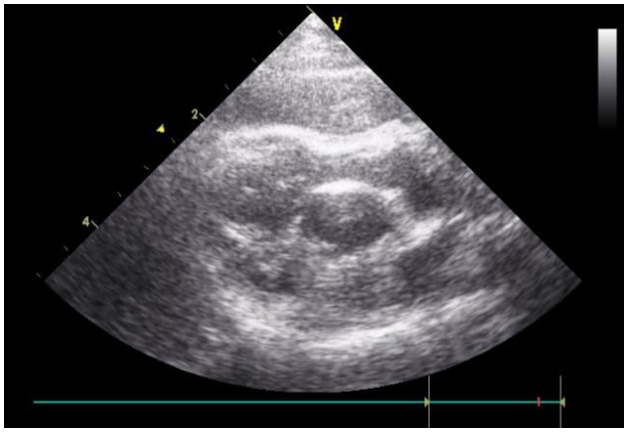

Fig 2. Five chamber view of heart in echocardiography.

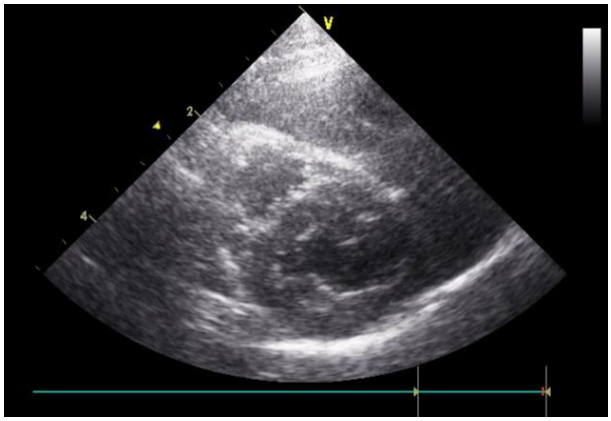

Fig 3. Dilation of both atrium and thickening of the muscular layer of the left ventricle in echocardiography.

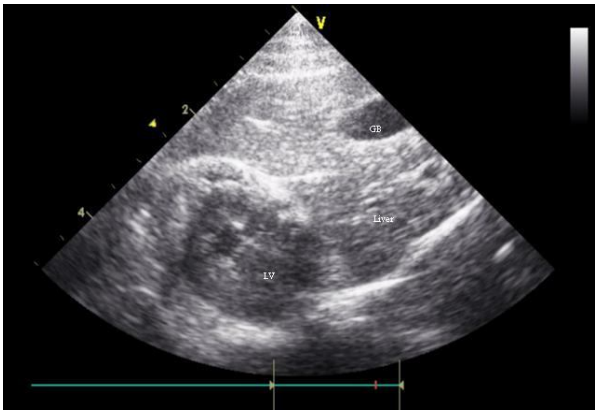

Fig 4. A view of heart, liver, and gall bladder in echocardiography.

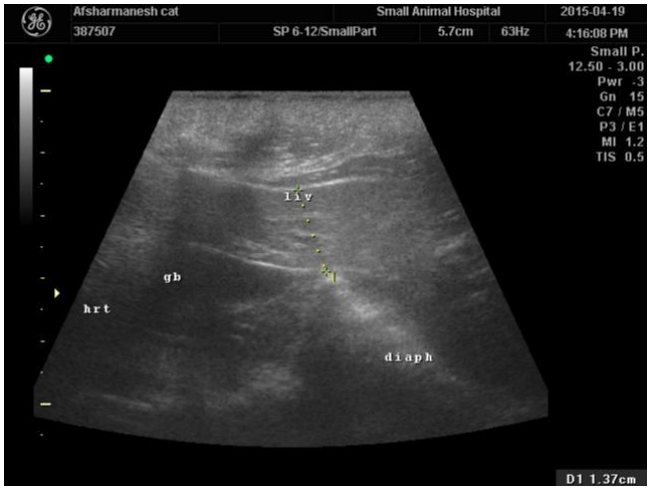

Fig 5. Diaphragmatic defect and liver herniation in ultrasonography.

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Genetic Diversity of Giardia Duodenalis Isolate from Cattle in Urmia City by PCR-RFLP Method of Fragment of Glutamate Dehydrogenase (Gdh) Coding Gene.

Reza Asadi Isakan[1], Zarrintaj Valadkhani[2], Sadegh Rahbari[3]*, Nasser Hoghoogi Rad[3], Hossein Nahravanian[2] and Karim Mardani[4]

[1]PhD Student Parasitology, Islamic Azad University, Science and Research branch, Tehran, Iran.
[2]Assistant Professor, Dept. of Parasitology, Pasteur Institute of Iran.
[3]Department of Pathobiology, School of Veterinary Medicine, Islamic Azad University Research and Science branch, Tehran, Iran.
[4]Department of Food Industry, School of Veterinary Medicine, Urmia University, Urmia, Iran.

Received: 21 Mar 2015 Revised: 25 Apr 2015 Accepted: 24 May 2015

***Address for correspondence**
Sadegh Rahbari
Department of Pathobiology,
School of Veterinary Medicine,
Islamic Azad University Research and Science branch,
Tehran, Iran.

ABSTRACT

Giardia is a protozoan parasite that is observed in a wide range of vertebrates. Giardiosis as a common parasitic disease in humans and animals have been reported. The parasites in humans by food and water contaminated with cysts usually are transmitted. The aim of this study was to determine the Assemblages of Giardia lamblia by using PCR-RFLP method and glutamate dehydrogenase gene in human and cattle of Urmia city. In this study, 5 Giardia -positive stool samples were collected of cattle. Cysts were concentrated by formalin - ether method. Genomic DNA was extracted by freeze-thaw cycles followed by using QIAamp Stool Mini Kit. After DNA extraction, in order to amplify the 432 bp expected size of GDH gene, Nested-PCR method was used. Ultimately to evaluate the prevalence of giardia Assemblages, the specific restriction enzymes such as Hpy188, BspLI (NIaIV) and Hin (BsaHI) were used . Results showed that all the tested samples are Assemblage BIII. Due to the widespread livestock in Urmia city and the results obtained in this study, it is concluded that, the predominant species in domestic ruminants and humans is BIII.

Key words: Giardia, PCR-RFLP, GDHgene, Urmia, calve

INTRODUCTION

Giardia intestinalis which is so called giardia duodenalis and giardia lamblia has a worldwide diversity which infects a wide range of vertebrates like wild and domestic animals, and human (1,2,3). The importance of Giardia parasite, as a main cause of diarrhea, has been comprehensively studied in molecular genetics and evolutionary biology because of its wide diversity (4, 5). The life cycle of this parasite has two stages including the Trophozoite Flagellate in duodenum and infected cysts in host's body and are wasted via stool remaining for several weeks in medium (6,7). Based on molecular specificities, the giardia lamblia is classified into 8 assemblages (A to G) and the human is infected by A and B assemblages. The A assemblage includes two categories in which AI is related with human and animals and the AII is dealt with human exclusively. Although the assemblageB is especially for human, it has been observed in animals as well (8). Most observed subtypes of mentioned assemblagein human include AI, AII, BII, and BIV (1, 9). Several studies have done on giardia duodenalis emphasizing that it can be taken into account as a potential risk factor (10). Since the A and B assemblages have zoonotic in human and many mammals, the animals rule in human epidemiologic infections has not been completely understood. Thus, identifying the infection base using determination of parasite genotype through Glutamate dehydrogenase gene by PCR-RFLP can be a useful method to identify the Giardia lamblia (2).since the propagation pattern of this parasite is different in parts of country, the present work deals with the evaluation of frequency of giardia duodenalis and the probable relation between the giardia assemblages and identification of parasite bases.

MATERIALS AND METHODS

Sampling: the sampling was done in Livestock around Urmia and the samples were examined by microscopic methods in Azad University. As the infection confirmed, the cysts were separated using formalin ether and kept in -20 ℃ till the DNA extraction. Among 30 positive separated samples, only 8 of them included a considerable amount of cyst.

DNA extraction

The concentrated cysts were washed with TE buffer twice and put into a 1.5 ml microtube. Then, a 500 µl of lysing buffer and the same volume of glass beads (12 beads) were added to microtube and vortexed for 10 min (1, 3). The DNA extraction was done based on 10 steps of fusion and freezing in which the 95 ℃ Water bath and liquid nitrogen was used for fusion and freezing respectively. Then, the QIAamp Stool Mini Kit was used to extraction according to the product factory recipe and the extracted DNA was kept in -20 ℃ till PCR.

PCR reaction

The glutamate dehydrogenase gene multiplication was carried out based on Nested-PCR method and a fragment of 432 bp was multiplicated from the following primers:

Forward (GDHFO):5' TYA ACG TYA AYC GYG GYT TCC GT 3'

Forward (GDHFI):5' CAG TAC AAC TCY GCY CTC GG 3'

Reverse (GDHR): 5' GTT RTC CTT GCA CAT CTC C 3'

the reaction contents in a 20 µl includes the Mgcl2 1.5 mmol.lit-1 , dNTP 200 µmol.lit-1, each primers 10 picomol.lit-1, Taq polymerase enzyme 1 unit and DNA 1.5 lambda. The Biorad thermocycler apparatus acts in in such a way that

each denaturation occur during 5 min at 94 ℃, 35 cycles of 45 seconds duration at 94 ℃, linking during 45 seconds at 94 ℃ , 35 cycles, elongation during 45 seconds at 72 ℃, and finally a long time elongation during 7 min at 72 ℃.

RFLP

The RFLP enzyme acts through digestion of 10 μl of PCR product , 1-2 μl of BspLI (NIaIV) and Hin(BsaHI), 2 μl of 10X enzyme and 18 μl of distilled water being incubated for 3 hours at 37 ℃ and kept in 65 ℃ for 20 min to denature the enzyme. For Hpy188I enzyme, 10 enzyme units are mixed with 5 μl of buffer 10X enzyme with 1 μg of PCR product reaching the final volume of 50 μl being incubated for an hour at 37 ℃ and kept in 65 ℃ for 20 min to denature the enzyme. The digestion enzyme BspLI(NIaIV) was used to identify the AI and AII assemblages, B,C,E, and F groups. The Hin(BsaHI) was used to identify the A,BII, and F assemblages. Finally, the Hpy188 enzyme was used to identify the BI, BII, BIV, C, and E assemblages.

RESULTS

In the present work, the DNA extraction was operated successfully for only 8 samples among 30 positive samples in which the considered 432 bp was observed in only 4 samples. The electrophoresis results of samples on gels are shown in Figure 1. The selective restriction enzimes Hpy188I, BspLI(NIaIV), and Hin(BsaHI) were used to identify the giardia parasite genotypes and the cut DNA by each enzyme is shown in table (1). All digested products by mentioned enzymes were transferred on a gel as shown in Figure (2). According to table (10, all samples showed the BIII assemblage.

DISCUSSION

The present study has been done to investigate the Giardia lamblia genetic diversity in cattle's of Urmia province and the bond was observed in 4 samples among 8 positive samples which all isolates showed assemblage BIII using PCR-RFLP.

Since there has been no report on identification of Giardia lamblia in calf and the A,B, and E assemblages have been reported in studies of the other parts of the world, all dominant assemblages is studies are E which is not in agreement with our results. The studies of Giardia genotype identification in human in Iran have shown the AI, BII, and BIV and the present work has most correspondence with the findings reported by Manafi et al. Souza et al, 2007, have studied the molecular identification of giardia duodenalis in human, cattle, and cat and observed 19 AII, and 8 B isolates among 37 human samples. Amon 19 cat samples, 11 F and 8 AI isolates have been reported. Also, they have observed 4 E and an AI isolates amon 5 cattle samples (1). Lalle et all have investigated the heterogenic study of human and livestock samples using β - giardin in Italy in which the assemblage of 37 human samples were determined that 16 case of A and 10 cases of B assemblages were observed and 11 cases showed both assemblages. Also, 6 A, a C, and 13 C isolate assemblages were observed among 21 isolate dogs and an isolate had both A and D genotypes. The assemble age of cat samples was determined using glutamate dehydrogenase in which all genotype isolates were reported F. Also, 12, 5, and 3 isolates showed the A, B, and E genotypes respectively, among 12 cattle samples. The sub genotypes of AI, AII, AII, AIV, and BIII were observed in infections related to human, dog, and cattle. Furthermore, the AI genotype role as the zoonosis clearly (9). Sarkari et al have studied the determination of giardia genotypes in 2012 in Shiraz city in which 74.41 %, 17.44%, and 3.49 % of samples belonged the AII, BIII, and BIV, respectively while 4.66 % of samples showed both AII and BIV genotypes (11). Fallah et all have investigated the genetic determination of giardia and 41.9% and 54.8% of samples showd B and A assemblages respectively while 3.2 % of samples showed both assemblages (12). Gelanew et al (2007) have identified the human isolates of giardia duodenalis molecularly using β - giardin in which 31 (52%) and 13(22%) isolates showed the A and B assemblages,

respectively and a significant relation between B assemblage infection and medical symptoms were reported. Also, 15(25%) of remained isolates were infected with both A+F and A+B in 7 and 8 isolates respectively which the F assemblage was confirmed in 3 isolates using sequencing of A+B isolates (13). The identification of giardia genotypes was done by Manafi et all,2013, in Urmia city in children confined in Urmia hospitals and 93.3%, 6.7% of samples showed the BII and BIV assemblages respectively (14). Geurden et al have aimed the identification of giardia genotypes of cattle in Belgium at 2008 in which 64% of studied castles (dairy and beef) showed the E isolate while 59% of dairy cattle showed the A assemblages (15). Soliman et al have identified the human isolates in Egypt at 2011 and the B assemblage was reported as the dominant one while the A and C assemblages were reported only in 1 sample (16). In an investigation in 2008, Garcia et al have identified the giardia genotypes in human and dog in which 52.6% and 47.4% of samples showed AI and AII respectively, in dogs while 41.7% and 58.3% of assemblages were AI and AII respectively, in human samples (17). Akbarian et al have aimed the genetic differences in Khoramabad city and suburban regions using PCR and sequencing in which all samples showed genotype A and no significant difference was observed among them. This study showed that the A assemblage dominant is in mentioned areas (18). Amer et al have studied the giardia phylogenetic in human and dairy cattle in Egypt in which A assemblage was observed in 37% and 22% of human and dairy cattle respectively. Also, it was reported that the dominant assemblage A was AII and AI in human and animal, respectively. The B and E were reported as dominant assemblages in human (66%) and animals (77.8), respectively indicating the heterogeneous genetic of all giardia genotypes which is attributed to the genetic diversity of giardia in Egypt emphasizing on the calves as the main causes of zoonotic giardia infection (19). Identifying the genotype diversity of giardia in England livestock in 2012, Minetti have found that E is the dominant among A and E observed assemblages and the infection related of both of them was reported as well. It is noteworthy that some cattle, sheep, and pigs were infected by C, D, and F assemblages unexpectedly. The pre-weaned calves showed a higher infection compared to adult ones while no significant relation was observed between the duodenalis infection risk and Giardia. The assemblage A prevalence and unordinary findings of giardia genotypes in unconventional hosts indicate that the multi Locus analysis should be used to verify the real diversity of Giardia duodenalis in future.Also, these data show that the medical importance of Giardia duodenalis should be evaluated again in livestock infection (20). Geurden et al have done the molecular identification and analyzed the risk factor in Italy, France, Germany, and England in 2012 indicating that the E assemblage is dominant. Although 43% of species showed the assemblage A in some countries, 32% of species showed both A and E assemblages which was in agreement with previous findings. The results of this work show that the giardia infection in calves has been more than two years before in European countries and the infection risk is decreased as the age increase (21). Aiming the genetic identification of Giardia duodenalis in dairy cattle in Sao Paolo, Paze Silva et al have done an investigation using GDH and SSU-rDNA genes and observed the giardia in 8 farms (80%) among 10 farms in which 15 cows were infected among total 200 dairy cows and the E and AI assemblages were observed in 14 (93%) and 1(7%) respectively. The aforementioned study shows that the uncommon E assemblage is current in the region and hence the zoonotic giardia infected cows can potentially infect the human and take into account as a risk for public health (22). Uehlinger et al have studied the giardia genotype of Canada in 2012 and reported that the prevalence rate are 40% and 27% in dairy and beef cattle respectively. The dominant assemblage E and zoonotic giardia genotype were observed in 12.2% of calves which is a risk for public health (23). The identification of giardia genotype in pre-weaned calves was studied by Santin et al in 2012. They observed the positive giardia of E and A assemblages in 31.7 and 1.2 % of species, respectively using molecular analysis. Also, the infection accompanied with both E and A assemblages was reported in 4 calves. These findings show that most giardia genotypes in pre-weaned calves are E types while the A assemblage is a potential risk for human population in minority of animals (24). Khan et al have evaluated the cattle importance as a main source of giardia infection in India in 2011and considered the prevalence of cattle Giardiasis. They have examined the 180 stool samples of dairy and dairy workers in west Bengal and analyzed them using β - giardin gene analysis and sequencing of PCR products showing a prevalence rate of 12.2%(22/180). The common genotype of human and livestock was reported AI in calves and workers while the most genotype was E. the mentioned findings indicate a potential risk of common infection between human and livestock in Indian diaries (25). Determining the frequency of giardia genotypes in new born and 2 years age calves, Santin et al have studied the prevalence rate of calves in 2009 and found out that the

highest infection was in 4-5 weeks old calves. The calves smaller than 8 weeks, 3-12 months old, and 12-24 months calves had highest prevalence rates of 60.8%, 32.1%, and 11.4% respectively. The sequencing analysis of species showed both A and E assemblages. No assemblage A was extracted from not pre-weaned calves while 9.6% of 3-12 and 4.7% of 12-24 moths age showed such genotype. These data show that not only the calves are infected with both A and E assemblages simultaneously, but the common infection risks of human and livestock is more prevalent than what had been reported previously. Thus, it seems that the calves are a noticeable source of infection for human (26). Foroda et all in 2008 have evaluated the giardia genotype in human in Egypt in which the sequencing analysis of human samples showed the B,E, and A assemblages having 80%,15%, and 5% respectively. Although B and E are conventional assemblages in human, the E genotype has been reported for the first time in human which necessitate a comprehensive study in this region (27). Winkworth have identified the giardia in calves and human in a region in New Zealand in which the diaries are widespread in 2008. They analyzed 40 cattle and 30 human samples using β – giardin method and both A and E assemblages were identified while no E assemblage have been reported in livestock (28). Itagaki et al have studied the domestic and wild animals aiming the genotyping giardia in Japan for the first time in 2005 and sampled 24 dogs, 3 cats, 5 cows, and 3 monkeys. They observed the A,C, and D assemblages in dogs, E assemblage in cats, A and E assemblages in cattle, and B assemblage in monkeys (29). Trout et al have determined the frequency of giardia genotype in 1-7 weeks age calves in 2004 in which the prevalence rate varied from 9% in a farm in Pennsylvania to 93% in a farm in Vermont and the average prevalence was reported 40%. The sequencing analysis using TPI, β – giardin, and 16-SrRNA genes showed that 85% and 15% of species are infected by E and A assemblages. Although most calves were infected by uncommon genotype with human, the calves of 7 farms (among 14 farms) were infected by A assemblage which is common between human and livestock. Thus, the calf can be a potential risk for human infection (31, 32).

Regarding that the livestock is very current in Urmia and based on the findings of this work, which indicate that the dominant sub genotype is BIII and is prevalent in ruminant domestic animals and human, it is concluded that the calf is the main cause of infection in this region.

ACKNOWLEDGMENTS

The authors are especially thankful for Pasteur Institute of Iran (IPI) and Gahamilooyi for their help in molecular studies and for Esmaeil Valizadeh, the parasitology laboratory expert of Islamic Azad University , Urmia branch.

REFERENCES

1. Souza, S.L.P., Gennari, S.M., Richtzenhain L.J., Pena, H.FJ., Funada, M.R., Cortez, A., Gregori, F., Soares, R.M. (2007). Molecular identification of Giardia duodenalis isolates from humans, dogs, cats and cattle from the state of São Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (gdh) coding gene. Veterinary Parasitology 149:258-64
2. Read, C., Monis, P., Andrewthompson, R. (2004). Discrimination of all genotypes of Giardia duodenalis at the glutamate dehydrogenase locus using PCR-RFLP. Infection, Genetics and Evolution 4:125-30
3. Babaei, Z., Oormazdi, H., Rezaie, S., Rezaeian, M., Razmjou, E. (2011). Giardia intestinalis: DNA extraction approaches to improve PCR results. Experimental Parasitology 128:159-62
4. Thompson, RC,. (2004). The zoonotic significance and molecular epidemiology of Giardia and giardiasis. Veterinary Parasitology 126:15-35
5. Thompson, RC. (2003). Giardiasis as a re-emerging infectious disease and its zoonotic potential. International Journal for Parasitology 30:1259-67
6. Amar, C., Dear, P., Pedraza-Diaz, S., Looker, N., Linnane, E., McLauchlin, J. (2002). Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of Giardia duodenalis in human feces. Journal of clinical microbiology 40:446-52

7. Eckmann, L., Gillin, FD. (2001). Microbes and microbial toxins: paradigms for microbial-mucosal interactions I. Pathophysiological aspects of enteric infections with the lumen-dwelling protozoan pathogen Giardia lamblia. American journal of physiology Gastrointestinal and liver physiology 280:G1-6
8. Monis, PT., Andrews, RH., Mayrhofer, G. (2003). Genetic diversity within the morphological species Giardia intestinalis and its relationship to host origin. Infection, Genetics and Evolution 3:29-38.
9. Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D., Cacciò, SM. (2005). Genetic heterogeneity at the β-giardin locus among human and animal isolates of Giardia duodenalis and identification of potentially zoonotic subgenotypes. International Journal for Parasitology 35:213-07
10. O'Handley, R., Olson, M., Fraser, D., Adams, P., Thompson, R. (2000). Prevalence and genotypic characterisation of Giardia in dairy calves from Western Australia and Western Canada. Veterinary parasitology 90:193-200
11. Sarkari, B., Ashrafmansori, A., Hatam, GR., Motazedian, MH., Asgari, Q., Mohammadpour, I. (2012). Genotyping of Giardia lamblia isolates from human in southern Iran. Tropical biomedicine. 29:366-71
12. Fallah, E., Nahavandi, K., Jamali, R., Mahdavi, B., Asgharzadeh, M. (2008). Genetic Characterization of Giardian intestinalis strain from Patients Having sporadic Giardiasis by using PCR Assey. Peer-reviewed scientific Journal 8:310-315
13. Gelanew, T., Lalle, M., Hailu, A., Pozio, E., Cacciò, SM. (2007). Molecular characterization of human isolates of Giardia duodenalis from Ethiopia. Acta Tropica Journal 102:92-9.
14. Manafi, G., Hazrati tappeh, K., Diba, K., Mohammadzadeh, H., Asgharzadeh, M., Gheibi, S. (2013). Evaluation of Frequency of Giardia lamblia sub Species by PCR-RFLP in hospitalized children, stool specimens in Urmia Mutahhari Hospital. Urmia Medical Journal 24:414-22
15. Geurden, T., Geldhof, P., Levecke, B., Martens, C., Berkvens, D., Casaert, S., Vercruysse, J., Claerebout, E. (2008). Mixed Giardia duodenalis assemblage A and E infections in calves. International Journal for Parasitology 38:259-64
16. Soliman, RH., Fuentes, I., Rubio, JM. (2011). Identification of a novel Assemblage B subgenotype and a zoonotic Assemblage C in human isolates of Giardia intestinalis in Egypt. Parasitology International 60:511-517.
17. Eligio-García, L., Cortes-Campos, A., Cota-Guajardo, S., Gaxiola, S., Jiménez-Cardoso, E. (2008). Frequency of Giardia intestinalis assemblages isolated from dogs and humans in a community from Culiacan, Sinaloa, Mexico using β-giardin restriction gene. Veterinary Parasitology 156:205-9.
18. Akbarian, A., Sadraie, J., Forozandeh, M. (2012). Evaluattion of Giardia lamblia genetic differences in Khorramabad City and surrounding villages by use of PCR and sequencing. Scientific Journal of Kurdistan University of Medical Sciences 17:61-71
19. Amer, SE. (2013). Genotypic and phylogenetic characterization of Giardia intestinalis from human and dairy cattle in Kafr El Sheikh Governorate, Egypt. Journal of the Egyptian Society of Parasitology 43:133-46
20. Minetti, C., Taweenan, W., Hogg, R., Featherstone, C., Randle, N., Latham, SM., Wasting, JM. (2013). Occurrence and Diversity of Giardia duodenalis Assemblages in Livestock in the UK. Transboundary and emerging diseases 10. 12075
21. Geurden, T., Vanderstichel, R., Pohle, H., Ehsan, A., von Samson-Himmelstjerna, G., Morgan, ER. (2012). A multicentre prevalence study in Europe on Giardia duodenalis in calves, with molecular identification and risk factor analysis. Vet Parasitol 190:383-90
22. Paz e Silva, FM., Lopes, RS., Araujo, JP. (2012). Genetic characterisation of Giardia duodenalis in dairy cattle in Brazil. Folia parasitologica 59:15-20
23. Uehlinger, FD., Greenwood, SJ., O'Handley, R., McClure, JT., Coklin, T., Dixon, BR., De boer, M,. Zwiers, M,. Barkema, HW. (2011). Prevalence and genotypes of Giardia duodenalis in dairy and beef cattle in farms around Charlottetown, Prince Edward Island, Canada. The Canadian veterinary journal La revue veterinaire canadienne 52:967-72
24. Santin, M., Dargatz, D., Fayer, R. (2012). Prevalence of Giardia duodenalis assemblages in weaned cattle on cattle-calf operations in the United States. Vet Parasitol 183:231-6
25. Khan, SM., Debnath, C., Pramanik, AK., Xiao, L., Nozaki, T., Ganguly, S. (2011). Molecular evidence for zoonotic transmission of Giardia duodenalis among dairy farm workers in West Bengal, India. Vet Parasitol 178:342-5

26. Santin, M., Trout, JM., Fayer, R. (2009). A longitudinal study of Giardia duodenalis genotypes in dairy cattles from birth to 2 years of age. Vet Parasitol 162:40-5.
27. Foronda, P., Bargues, MD., Abreu-Acosta, N., Periago, MV., Valero, MA., Valladares, B., Mas-Como, S. (2008). Identification of genotypes of Giardia intestinalis of human isolates in Egypt. Parasitology research 103:1177-81
28. Winkworth, CL., Learmonth, JJ., Matthaei, CD., Townsend, CR. (2008). Molecular characterization of Giardia isolates from calves and humans in a region in which dairy farming has recently intensified. Appl Environ Microbiol 74:5100-5
29. Itagaki, T., Kinoshita, S., Aoki, M., Itoh, N., Saeki, H., Sato, N., Uetsuki, J., Izumiyama, S., Yagita, K. (2005). Genotyping of Giardia intestinalis from domestic and wild animals in Japan using glutamete dehydrogenase gene sequencing. Vet Parasitol 133:283-7
30. Matsubayashi, M., Kimata, I., Abe, N. (2005). Identification of genotypes of Giardia intestinalis isolates from a human and calf in Japan. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 67:337-40
31. Trout, JM., Santin, M., Greiner, E., Fayer, R. (2004). Prevalence of Giardia duodenalis genotypes in pre-weaned dairy calves. Vet Parasitol 124:179-86
32. Olfati A, Moghaddam GH, Moradi Kor N, Bakhtiari M. 2014. The Relationship between Progesterone and Biochemical Constituents of Amniotic Fluid with Placenta Traits in Iranian Crossbred Ewes (Arkhar-Merino×Ghezel). Asian Pacific Journal of Tropical Medicine. 7 (Suppl 1): S162-S166.

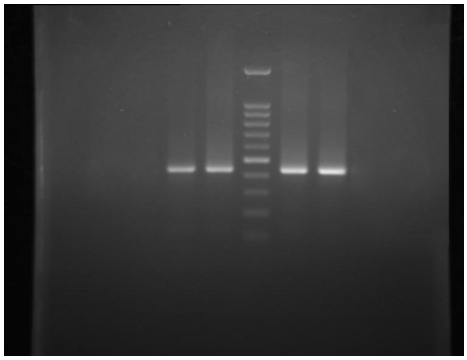

Figure (1): the electrophoresis results of PCR products on agar gel (1.5%)

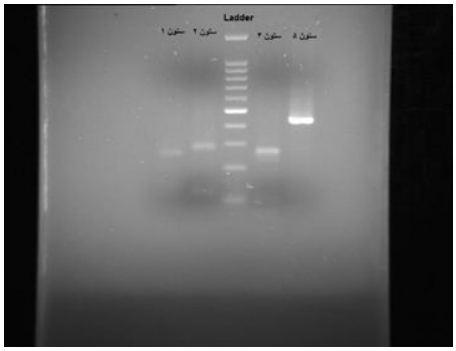

Figure (2): PCR-RFLP of restriction enzymes including Hyp1881, NLaIV, bp marker 100,

Table (1): size of fragments cut by each restriction enzyme

Sub Genotype	Enzyme Genotype	Diagnostic Fragment
BII	BspL1 Hyp188I Hin1I	287,123,22 148,24,202,58 264,66,102
BIII	BspL1 Hyp188I Hin1I	287,123,22 121,25,24,262 264,66,102

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Ethno Pharmacological Survey and Introduction of Medicinal Plants of Rykan in the Northwest (Urmia, Iran)

Leila Mirzaei[1]* and Hanieh Moradbeigi[2]

Young Researcher and Elites Club, Islamic Azad University, Urmia Branch, Urmia, Iran.

Received: 25 Mar 2015 Revised: 22 Apr 2015 Accepted: 29 May 2015

***Address for correspondence**
Leila Mirzaei
Young Researcher and Elites Club,
Islamic Azad University,
Urmia Branch, Urmia, Iran
Mobile: +989144391957.
E-mail: l.mirzaei_2009@yahoo.com,

ABSTRACT

Iran with 1.64 million km^2 areas has 8000 plant species, that extensive experience the use of herbal plants.Iran because of the weather and ecological conditionspossesses enormous diversity flora. This study documents 84 medicinal plant species were collected and identified in Rykan region (Urmia Province), northwest, Iran. The botanical name, family name, partused of the plants has been provided in this paper. The plants were gatheredbelonged to 29 different plant families. Asteraceae (14%),Lamiaceae (13%), Fabaceae (10%) families had the highest frequency. Based our results the most common parts used as medicinal sections were flowers (23%), leaves (21%) andUse of the bulb (4%), Sap and bark (1%) were lower than the others. The purpose of this study is gathering and identifying medicinal plant with aims at emphasizing the greatest importance of investigation of pharmacological study**.**

Key words: Iran, flora, secondary metabolites, natural drugs, medicinal plants.

INTRODUCTION

According to research carried out, more than 422000 species of flowering plants have been reported from all over the world which 5000 species among them are used for medicinal purposes (Mozaffarian,2005). These plants play an essential role in traditional health care, about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.Natural sources have been the source of medicinal agents for thousands of years, and some important modern drugs have been derived from natural sources, many based on their use in traditional medicine. Iran has one of the most enriched floras of the world that have caused a wide distribution of medicinal plant species (Akhundzadeh, 2000; Azadbakhat, 1999; Mousavi, 2004; Zargari, 1997). Iranians have been using herbal

medicine for the treatment of some daily diseases. Properties of medicinal plants are due to different composition which named secondary metabolites (alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate). Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids and essential oils. All extracts and chemical compounds of plants to produce human drugs or veterinary medicine (Javanmardi et al, 2003; Nikbakht et al, 2008; Kiran et al, 2006; Raghavendra et al, 2006; Yousefzadiet al, 2011). Moreover some of the Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, agroalimentary and cosmetic industries (Ghasemi Pirbalouti et al,2009; Najafi et al, 2010 ; Rhaman et al, 2004; Khorasaninejad et al, 2010). Essential oils extracted from the medicinal plants, used as antimicrobial agents in food systems may be considered as an additional intrinsic determinant increase the safety and shelf life of foods (Nejad Ebrahimi et al, 2008). Today, plant materials continue to play a major role in primary health care as therapeutic remediesin. many developing countries and because of their harmful and irreversible effects on people, are being replaced by a substances extracted from medicinal plant (Nezhadali et al, 2010) .The use of medicinal plant s has been widely promoted and statistics obtained show that more than 3.3 billion people of the world utilize medicinal plants on a regular basis .So, identification and preservation of these valuable resources are necessary (Hamilton, 2003).The our study attempts to identify native medicinal plants and use them.

MATERIALS AND METHODS

The studied region is located in SW of West Azerbaijan province.The study area is 8 km from the city of Urmiathat located at 35-39 to 44-47 eastern latitudes and 31-23 to 31-27 northern longitudes widths.The average yearly rainfall is 300 millimeters. Annual minimum and maximum temperature average are 16°C and 32°C, respectively. First of all the basic information in this study is gathered and then some visit from the region is done. The collected plant taxa were dried according to herbarium techniques, and numbered then recorded. The Flora of Iranica (Rechinger, 1998-2005), Flora Orientalis (Boissier ,1936), Flora of Iraq (Townsen et al, 1985), Flora of Turkey (Davis, 1965– 1985), Flora of Iran (Asadi et al, 1988-2011), Colored Flora of Iran (Ghahreman, 1977–2007) and other applicable resources and references were used for the identification of the specimens. The ethnomedicinal inventory is presented by plant name, family and used parts (Table 1).The Diagram of abundance was designed by EXCEL and the frequency of drug plant families was calculated. Collected species using botany cited sources identified and then in terms of the value of medicine as well as the match with resources and eventually species and parts of pharmaceutical companies to be used as a herbal medicine (Table1).

RESULTS

Iran is an ancient country in usage of herbal plants and there are documents showing Persians were pioneers in applying plants for medicinal purposes (Nikbakht et al, 2004). Iran has 7500-8000 plant species (Rechinger, 1982). Despite the vast knowledge of medicinal plants existed in Iran, a few attempts have been carried out to document ethnobotanical knowledge. . Some researchers have investigating the plants in different areas of the country (Amiri, 2012; Emami et al,2012; Ghorbani, 2005; Mosaddegh et al, 2012; Naghibi et al, 2005; Rajaei, 2012; Safa et al, 2013; Zargari, 1989-1992). Most of the reported species have not been studied for their chemical constituents' andor biological activities. This study aims at emphasizing the greatest importance of investigation of those species that havenot been the subject of pharmacological and chemical studies, although their popular uses have been reported.

This study provides information on 83 medicinal species belonging to 29 families that are most commonly used for traditional medicine in Rykan region. Botanical names of plants were sorted alphabetically, and for each species and the following information was hence represented: family, genus, part used (Table 1). Results showed that species belonged to 32 families. Asteraceae (14%), Lamiaceae (13%), Fabaceae (10%) and Brassicaceae (9%) families had the

highest frequency that this plant family is the most important families in medicinalplants. Rubiaceae(2%), Malvaceae(2%), Plantaginaceae (2%) family had the lowest frequency (Figure 1).

As well as several plant families with a known species.Different parts of medicinal plants were used by the inhabitants of Rykan region as medicine for treating ailments. In this study different organs with different medicinal value of plants have been identified wich in presented in Figure 2. Based our results the most common parts used were flowers (23%), leaves (21%). Use of the bulb (4%), Sap and bark (1%) were lower than the others.

CONCLUSION

Identify medicinal plant and traditional uses of these plants providing useful information on the distribution and field provides for other pharmaceutical activity in relation to this topic. High ecological diversity and the public on a broad approach to the use medicinal plants on the other hand represent the necessary extensive research on medicinal plants in the world.

ACKNOWLEDGMENTS

Authors are thankful to Young Researcher and Elite club (Islamic Azad university Urmia branch) for providing financial assistance in the form of a major research project. Special thanks to Dr.Ashkan Khoda bandehlou, for helping me with my experiments.

REFERENCES

1-Akhundzadeh P. 2000. Encyclopedia of Medicinal Plants of Iran. Institute of Medicinal Plants of the University jahad.
2-Amiri MS, Jabbarzadeh P, Akhondi M. 2012. An ethnobotanical survey of medicinal plants used by indigenous people in Zangelanlo district, Northeast Iran. J Med Plants Res, 6: 749-753.
3-Assadi M, Maassoumi AA, Khatamsaz M and Mozaffarian V. 1990 – 2010. Flora of Iran (In Persian). Research Institute of Forests and Rangeland Press. Tehran.
4- Azadbakhat M. 1999. Classification of medicinal plants. Cultural Institute Tymoorzadeh publication.
5-Boisseir E. 1867 – 1888. Flora Orientalis, A. Asher and Co. B. V. Amsterdam.
6- Davis PH. (ed.) .1965 – 1985.Flora of Turkey. Edinburgh University Press, Edinburgh.
7- Emami SA, Nadjafi F, Amine GH, Amiri MS, Khosravi Mt, Nasseri M. 2012. Les especes de plantes medicinales utilisees parles guerisseurs traditionnels dans la province de Khorasan, nord-est de l'Iran. J Ethnopharmacol, 48: 48-59.
8- Ghahraman A. 1975-2003. Colored Flora of Iran.Research Institute of Forests and Rangelands Press, Tehran (In Persian).
9- Ghasemi Pirbalouti A, Koohpayeh A, Karimi I. 2010. The wound healing activity of flower extracts of Punica granatum and Achillea kellalensis in wistar rats. Acta Poloniae Pharmaceutica - Drug Research. 67: 1070-1110.
10- Ghorbani A. 2005. Studies on pharmaceutical ethnobotany in the region of Turkmen Sahra, north of Iran (Part1): General results. J Ethnopharmacol, 102: 58-68.
11- Hamilton A. 2003. Medicinal Plants and Conservation, International Plants Conservation Unit, Panda House, Catteshall Lane Godalming, UK.
12- Javanmardi J, Stushnoff C, Locke E, Vivanco JM. 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chemistry. 83: 547–550.
13- Kiran B, Raveesha KA. 2006. Antifungal activity of seed extract of Psoralea corylifolia L. Plant Disease Research. 20: 213-215.

14- Khorasaninejad S, Mousavi A, Soltanloo H, Hemmati Kh, Khalighi A. 2010.The Effect of Salinity Stress on Growth Parameters, Essential oil Yield and Constituent of Peppermint (Mentha piperita L.). World applied Sciences Journal. 11: 1403-1407.

15- Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaeili S. 2012. Ethnobotanical survey of herbal remedies traditionally used in Kohgiluyeh va Boyer Ahmad province of Iran. J Ethnopharmacol, 141: 80-95.

16- Mozaffarian V. 2005. Getting to know medicinal plants and the problems associated with them. Proceeding at the National Conference on Sustainable Development of Medicinal Plants. Mashhad.

17- Mousavi A. 2004. Herb Zanjan, Iran Medicinal and Aromatic Plants Research Quarterly. 20. (3): 368-345.

18- Naghibi F, Mosaddegh M, Mohammadi Motamed S, Ghorbani A. 2005. Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. Iran J Pharm Res, 4: 63-79.

19- Najafi Sh, SadeghiNejad B, Deokule SS, Estakhr J. 2010 .Phytochemical screening of Bidaria khandalense (Sant.) Loranthus capitellatus Wall. Viscum articulatum burm.F and Vitex negundo Linn.Research Journal of Pharmaceutical, Biological and Chemical Sciences. 1: 388-393.

20- Nejad Ebrahimi S, Hadian J, Mirjalili MH, Sonboli A, Yousefzadi M. 2008 . Essential oil composition and antibacterial activity of Thymus caramanicus at different phenological stages. Food Chemistry. 110: 927-931.

21- Nezhadali A, Zarrabi Shirvan B. 2010 .Separation, Identification and Determination of Volatile Compounds of Ziziphora persica Bunge Using HS-SPME/GC-MS. Inter. J. Environ. Sci. Develop. 1: 115-118.

22- Nikbakht A,Kafi M, Haghighi M. 2008. The abilities and potentials of medicinal plants production and herbal medicine in Iran. Acta Horticulturea. 790: 259-262.

23- Rahman MA, Mossa JS, Al-Said MS, Al-Yahya MA. 2004. Medicinal plant diversity in the flora of Saudi Arabia1: a report on seven plant families. Fitoterapia 75: 149–161.

24- Rajaei P , Mohamadi N, Motamed M. 2012. Ethnobotanical Study of Medicinal Plantsof Hezar Mountain Allocated in South East of Iran. Iran J Pharm Res, 11: 1153-1167.

25- Raghavendra MP, Satish S, Raveesha KA. 2006. Phytochemical analysis and antibacterial activity of Oxalis corniculata: a known medicinal plant. My Science. 1: 72-78.

26- Rechinger KH. 1963-2009. Flora Iranica, Akademische Drucku Verlagsanstalt,Graz.

27- Safa O, Soltanipoor MA, Rastegar S, Kazemi M, Dehkordi Kh, Ghannadi A. 2013. An ethnobotanical survey on hormozgan province,Iran. Avicenna J Phytomed, 3:64-81.

28- Townsend C.C and Guest E. 1966 – 1985. Flora of Iraq. Minstry of Agriculture and Agrarian Reform, Baghdad, Iraq.

29- Yousefzadi M, Mirjalili MH, Alnajar N, Zeinali A, Parsa M.2011.Composition and in vitro antimicrobial activity of the essential oil of Dorema ammoniacum D. Don. Fruitfrom Iran. J. Serb. Chem. Soc. 76: 1-7.

30- Zargari A. 1989-1992. Medicinal Plants. Tehran University Publication, Tehran, 1-5: 4678.

31- Olfati A, Moghaddam GH, Moradi Kor N, Bakhtiari M. 2014. The Relationship between Progesterone and Biochemical Constituents of Amniotic Fluid with Placenta Traits in Iranian Crossbred Ewes (Arkhar-Merino×Ghezel). Asian Pacific Journal of Tropical Medicine. 7 (Suppl 1): S162-S166.

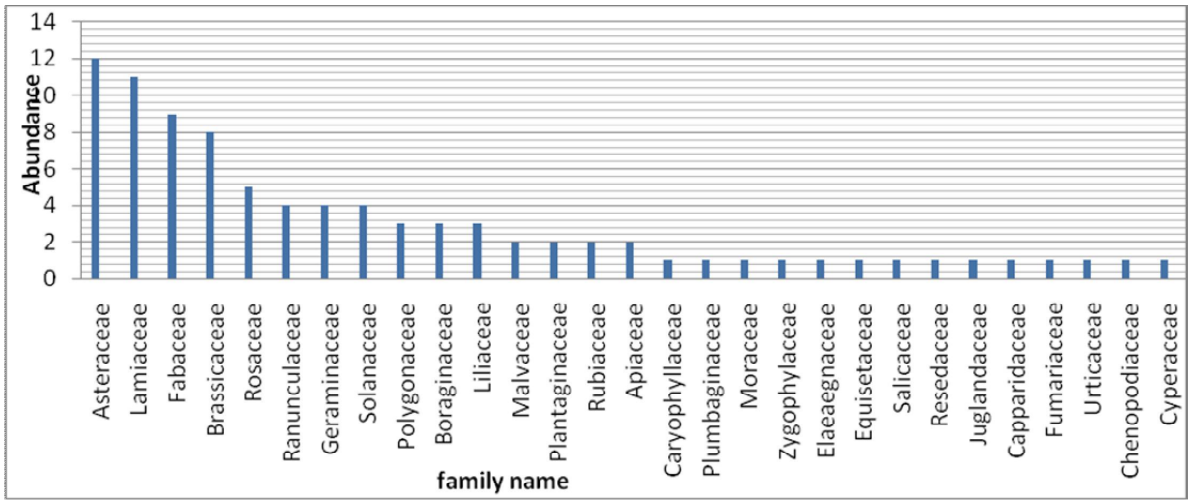

Figure1:Abundance of medicinal plant families in the region

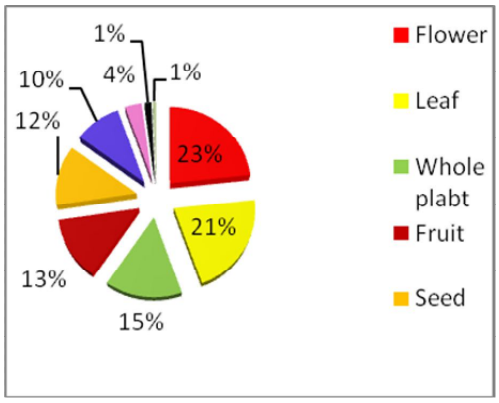

Figure 2. Plants part use and their percentage

Table1. Medicinal plants used by the traditional healers for the treatment of various ailments in Iran.

Part of plant used	Scientific name	Genus	Family name
All plant	A.aestivalis L.	Adonis	Rununculaceae
All plant	R.lomatocarpus D.C	Ranunculus	Rununculaceae
Leaf, flowering shoot	F. parviflora Lam.	Fumaria	Fumariaceae
Aerial organs	P.convolvolus L.	Polygonum	Polygonaceae
Root, Leaf, Seed	R. scutatus L.	Rumex	Polygonaceae
Leaf, Seed,flowering shoot	M. negelecta Wallr.	Malva	Malvaceae
Leaf, seed, flower	A.bracteatum Boiss. & Buhse.	Alyssum	Brassicaceae
Seed	C.bursa-pastoris(L.)Medik.	Capsella	Brassicaceae

Seed	D.sophia (L.) Schur.	Descurainia	Brassicaceae
Aerial organs	L.latifulium L	Lepidium	Brassicaceae
All plant	M .sativa L.	Medicago	Fabaceae
All plant	M .albus Medicus	Melilotus	Fabaceae
Root, Flower	S. alopecuroides L.	Sophora	Fabaceae
Flower	T.repens L	Trifolium	Fabaceae
Aerial organs	Z. tenuior L.	Ziziphora	Lamiaceae
Seed, Root	P. miliaceum L.	Panicum	Poaceae
Leaf	E.ciconium (L.) L Her.	Erodium	Geraniaceae
Leaf	F.vulgaris Bernh.	Falcaria	Apiaceae
Bark, Root, Fruit	L.ruthenicum Murry	Lycium	Solanaceae
Flower	A.bracteosa Boiss.	Alkanna	Boraginaceae
Flower	A. italica Retz.	Anchusa	Boraginaceae
Aerial organs	L. amplexicaule L.	Lamium	Lamiaceae
Leaf, flowering shoot,	M.longifolia L.	Mentha	Lamiaceae
flowering shoot	S.nemorosa L.	Salvia	Lamiaceae
Leaf, flower	S. lavandulifolia Vahl.	Stachys	Lamiaceae
Leaf, Fruit	P. lacceolata L.	Plantago	Plantaginaceae
Leaf, Seed,Root, Fruit	p. major L.	Plantago	Plantaginaceae
Flowering shoot	A.tenuifolia lam.	Achillea	Asteraceae
Flowering shoot	A. micrantha Wild.	Achillea	Asteraceae
Seed	C. tinctorius L.	Carthamus	Asteraceae
Aerial organs	C. depressa M.Bieb.	Centaurea	Asteraceae
Root, Seed, Basal leaf	C.intybus L.	Cichorium	Asteraceae
All plant, Sap	L.serriola L	Lactuca	Asteraceae
Aerial organs	T graminifolius DC.	Tragopogon	Asteraceae
Aerial organs	T. pratensis L.	Tragopogon	Asteraceae
All plant	C. dactylon (L.) Pres.	Cynodon	Poaceae
Flower, Leaf	A.officinalis L.	Althaea	Malvaceae
Flower, Leaf	A ampeloprasum L.	Allium	Liliaceae
Root, Rhizome	C. rotundus L.	Cyperus	Cyperaceae
Bark,Leaf,Fruit	J.regia L	Juglans	Juglandaceae
Bulb, Flower	F. persica L.	Fritillaria	Liliaceae
Fruit	Echinops ritro L.	Echinops	Asteraceae
Bulb	M. neglectum Guss. ex	Muscari	Liliaceae
Fruit,Root	D. carota L.	Daucus	Apiaceae
flower	S .schlschlegleevii Sosn.	Stachys	Lamiaceae
Aerial organs	E. sativa Lam	Eruca	Brasicaceae
Seed	C. draba (L.) Desv	Cardaria	Brasicaceae
Flowering shoot,Root	R. luteola L	Reseda	Resedaceae
Leaf, Flowering shoot	M. officinalis (L.) Desr	Melilotus	Fabaceae
Fruit,Leaf, Flower	S. alba L	Salix	Salicaceae
Aerial organs	G. verum L	Galium	Rubiaceae

Leaf	C.varia L.	Coronilla	Fabaceae
Aerial organs	. E. arvense L	Equisetum	Equisetaceae
Flower	N. persica Boiss.	Nonea	Boraginaceae
Root	R. tinctorum L.	Rubia	Rubiaceae
Fruit, Leaf, Seed	C.vulgaris Press.	Cydonia	Rosaceae
All plant	U. dioica L.	Urtica	Urticaceae
Seed	V. cracca L.	Vicia	Fabaceae
All plant	S. nigrum L	Solanum	Solanaceae
Seed	S. officinale L.	Sisymbrium	Brassicaceae
Fruit, Leaf, Flower	R.canina L.	Rosa	Rosaceae
Leaf , Flowering shoot	S. verticillata L.	Salvia	Lamiaceae
Leaf, Root or Rhizome	G. glabra L.	Glycyrrhiza	Fabaceae
Aerial organs	P .Polygonum aviculare L.	Polygonum	Polygonaceae
Fruit, Leaf, Flower	E. angustifolia L	Eleaegnum	Elaeaegnaceae
Leaf, Seed	D. stramonium L.	Datura	Solanaceae
Fruit, Leaf, Flower	A. communis L.	Amygdalus	Rosaceae
Leaf, Seed, Root	L.P. harmala	Peganum	Zygophylaceae
Fruit, Leaf, Flower	R. damascens Miller	Rosa	Rosaceae
Fruit, Leaf, Flower	F. carica L.	Ficus	Moraceae
flowering shoot, Seed	A .talyschensis A. Fedor	Anthemis	Asteraceae
All plant	P. europaean L.	Plumbago	Plumbaginaceae
flowering shoot	S. atropatana Bunge.	Salvia	Lamiaceae
Root	A. microcephalum Boiss.	Acantholimon	Caryophyllacea
Leaf, flower	Consolida oliveriana (DC.) Schrod.	Ranunculus	Ranunculaceae
Fruit	C. spinosa L.	Capparis	Capparidaceae
flowering shoot	A. millefolium L	Achillea	Asteraceae
flowering shoot	N. meyeri Benth.	Nepeta	Lamiaceae
Seed	H.reticulatus L.	Hyoscyam	Solanaceae
Aerial organs	N. Officinale (L.) R.Br.	Nasturtium	Brassicaceae
flowering shoot, Leaf	Teucrium polium L.	Teucrium	Lamiaceae
flowering shoot, Leaf, Fruit, Seed	P. sativum L.	Pisum	Fabaceae
Leaf ,f lower	A. wilhelmsii C. Koch.	Achillea	Asteraceae
All plant	R.arvensis L.	Ranunculaceae	Ranunculaceae
Aerial organs	C.botrys L.	Chenopodium	Chenopodiaceae
Leaf, Fruit ,Root	Ph. australis (Cav.) Trin.	Phragmites	Poaceae
Fruit	H. vulgare L.	Hordeum	Poaceae
flowering shoot	T. kotschyanus Boiss.	Thymus	Lamiaceae

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparison of Ultrasound-Assisted Extraction with Conventional Extraction Methods of Natural Antioxidants from Lettuce (*Lactuca sativa* L.) By-Products

Neda Ahmadi Kamazani[1], Amir Hossein Elhami Rad[1]*, Mehrdad Ghavami[2], Mahdi MoridiFarimani[3] and Mohammad Armin[4]

[1]Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran
[2]Department of Food Science and Technology, College of Food Science and Technology,Science and Research Branch, Islamic Azad University, Tehran, Iran
[3]Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Evin, Tehran, Iran
[4]Department of Agronomy and Plant Breeding, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran.

Received: 19 Mar 2015 Revised: 21 Apr 2015 Accepted: 27 May 2015

***Address for correspondence**
Amir Hossein Elhami Rad
Department of Food Science and Technology,
Sabzevar Branch, Islamic Azad University,
Sabzevar, Iran.
E-mail: ahelhamirad@yahoo.com

ABSTRACT

The agri-food by-products might be used as a source of substances with potentialsin pharmaceutical,food and cosmetics industries and the development of processes for their recovery results inagreat economic benefit for the agri-food producers and has a positive environmental impact.In this researchstudy, the effects of different extraction techniques, including ultrasound-assisted, maceration and soxhlet extraction, on the extractive yield, total phenolic contents (TPC) and DPPH-scavenging activityof lettuce by-productsextracts were investigated.The total phenolic contentsand the antioxidant activity of the 70 % ethanol extracts were determined according to the Folin-Ciocalteu method andthe DPPH assay, respectively.The effects of solvent to solid ratios (10:1, 20:1, 40:1, 80:1; v:w) over a 30 min extraction period, with a 70% ethanol solution, at 45°C on the yields, TPC and the antioxidant activitiyof the isolated extracts using ultrasound-assisted extraction were investigated.Different extraction methods showed significant differences in the extractive yield, TPC and the antioxidant activity.The highest extractive

yields,TPC and the antioxidant activitywere obtained by usingthe ultrasound-assistedextraction.In the ultrasound-assisted extraction a marked increase of the yield of extractwas observed when 20:1solvent–solid ratio was applied.The results showed that TPCwere increased by increasing the solvent to solid ratio.The highest antioxidant activity of extract was noticed when the solvent to solid ratio was at 20:1.The results indicated that ultrasound-assisted extraction technology improves the extraction ofbioactive compounds from lettuce(Lactuca sativa L.) by-productsboth qualitatively and quantitatively.

Key words: ultrasound-assisted extraction, conventional extraction methods, Lettuce by-products, antioxidant activitiy, total phenolic contents.

INTRODUCTION

Polyphenols constitute an important part of human diet. These bioactive compounds are mainly found in fruits and vegetables. The Possible healthful benefits of phenolic compounds consumption are the result of their antioxidant properties[31].Dietary antioxidants play a crucial role in human immune system protecting it from oxidative damage caused by reactive oxygen species involved in aging and many diseases [7]. Recent evidence strongly suggestspolyphenoles involvement in suppressing cancer chronic diseases including cancer, atherosclerosis, cardiovascular and central nervous system diseases as well as aging [21]. Fruit and vegetable production and processing leave substantial amounts of wastes and by-products. Wastes and by-products are commonly discarded, while provide a good source of antioxidant polyphenols[46,63]. Using these wastes as a source of Polyphenolsmight offer considerable economic benefits tofood processors. In addition, the antioxidant and cytoprotective activities of polyphenols existing in wastes and by-products are of utmost importance in demonstrating their potential healthful effects in human nutrition [30].Also the recovery of bioactive compounds from wastes and by-productsmight bequite effectiveinpreventing environmental pollution and might have a positive impact on this subject.

Lettuce (Lactuca sativa L.) is a leafy vegetable belonging to Asteraceae family which is consumed mostly as fresh or in salads due to its healthful nutritional benefits[16].Recently, the consumption of lettuce has increased worldwide due to its therapeutic effects. The healthful properties are attributed to high distribution of natural antioxidantssuch as ascorbic acid,tocopherols,carotenoids and polyphenols as well as fiber content[53,43,36] and otherphytochemicalsthat contribute to sensory characteristics and promote the health properties namelyanthocyanins and chlorophylls [33].Some researchers have indicated that lettuce by-products might be used as an interesting and inexpensive source of natural antioxidants forfunctionalization of foods [37]. There is a wide range of reports on the antioxidative effects of lettuce extracts[4, 5].Ozgen and Sekerci (2011)showedthat outer leaves of both red and green lettuce had higher total phenolics and antioxidant capacity than inner leaves however the outer leaves are usually removed prior to processing.Gomes et al.(2013)investigated the application of response surface methodology for obtaining lettuce(Lactuca sativa L.) by-products extracts with high antioxidative properties.

Conventional extraction methods such as soxhlet and maceration are still in use in spite of high solvent, time and energy consumption. Although over the past decades some novel extraction techniques including ultrasound and microwave-assisted extraction as energy saving and environment-friendly processes for low-cost and effective production of high-quality extracts have been developed [62].

Recently agricultural and industrial wastes such as grapes skin, grape seeds, almond hulls, buckwheat, fruit peels have been considered as main sources for producing valuable compounds [26, 1, 12, 14].Pan et al. (2012) demonstrated that UAE was an effective, reliable and feasible technique for the extraction of phenolic compounds from hawthorn seed with high yield as compared to classic extraction.Feng et al.(2015) investigated the ultrasound-assisted extraction and purification of phenolic compounds from sugarcane (Saccharum officinarum L.)

rinds.Ghitescu et al. (2015)found that an importantquantity of bioactive compounds might be extracted from spruce wood bark using ultrasound-assisted extractionwith 70% ethanol as the extracting solvent.Wong-Paz et al. (2015)studied ultrasound-assisted extraction of polyphenols from native plantsin the Mexican desert.

There are not reports on the application of ultrasound to extract theantioxidants from lettuce by-products.The aim of the current study was toinvestigate and compare the extractionyield, antioxidant activity and total phenoliccontents of thelettuce by-products extractsobtained by maceration (ME), soxhlet extraction (SE) andultrasound(UAE).The effect of solvent to solid ratio on the yield, TPC and antioxidant activity of the extracts obtained by the ultrasound-assisted extractionwas investigated sincesolid to liquid ratio is considered as a significant variable thatinfluences the extraction of polyphenols [41].

MATERIALS AND METHODS

Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Gallic acidwere purchased from SigmaChemical Co., Ltd (St. Louis, MO, USA). Folin-Ciocalteu reagent,butylated hydroxytoluene (BHT)were obtainedfrom Merck (Darmstadt, Germany). All the chemicals and reagents were of analytical and HPLC grades.

Plant material

A commercial variety of lettuce (Romaine) was used in thisstudy.The outer leaves of the lettuces which are commonly discarded by the sellers and consumers were separated and washed thoroughly with tap water. The leaves were cut into small pieces and dried at 60°C for a sufficient time [19] and then powdered by an electrical milland passed through a sieve with theproper mesh. The obtained powder was kept in a dark glass container at refrigerator (4°C) untilthe end of experimental period.

Extraction of plant material

Ultrasound- assisted extraction (UAE)

The powdered samples were extracted with EtOH/H_2O(70:30, v/v)usingultrasonic extraction at 30°C and 10×3 min. Extraction was performed at a solid to liquid ratio of 1:20 (w/v) in anultrasound waterbath at 40 kHz(WUC-D10H, Wiseclean, Korea). Theultrasonic bath was equipped with a digital thermometer and a recorder temperature and constant temperaturewas maintained throughout the work. A capped glass container was used in order to minimize solvent loss.

The samples were filtrated through Whatman No.1 filter paper after extraction andthe extractswere concentrated to dryness usingrotary evaporatorat 40°Cand the extractive yieldswere determined gravimetrically.The extracts were stored at 4°C in light-protected containers until required for the analysis of total phenolic contents and antioxidant activity.

Maceration

The powdered samples were extracted withEtOH/H_2O(70:30, v/v)over a magnetic stirrer (200 rpm) at 30°C (room temperature) and 24×3hour. Extraction was performed at a solid to liquid ratio of 1:20 (w/v).Separation and further treatment of the filtrates were carried out as described previously.

Soxhlet Extraction

The powdered sampleswere extracted with EtOH/H_2O(70:30, v/v)using soxhlet apparatus for a period of4 hourat a solid to liquid ratio of 1:20 (w/v).The liquid extract was evaporated by vacuum rotary evaporator at 40°C to dryness and the yield was determined gravimetrically.

Ultrasound-assisted extraction of antioxidantswith different solvent to solid ratios

Ultrasound-assisted extraction employingdifferent solvent to solid ratios were carried out at ratios of 10:1, 20:1, 40:1, 80:1; v:w for a 30 min extraction period using EtOH/H_2O(70:30, v/v)at 45°C.

Total phenolic contents

Total phenolic contentswere determined by Folin-Ciocalteumethod (56)with some modifications. Briefly, 25$\mu l$of extract was mixed with 125 μl of 1:10diluted Folin-Ciocalteureagent for 4 min, then 100 $\mu l$of saturated sodium carbonate solution was added. The mixture was incubated in darkness for 2h at room temperatureand the absorbance was determined at 760 nm. Gallic acid was used for calibration and the results were expressed as mg Gallic acid equivalents/100 g plant dry matter. A micro plate reader(Bio-Tek Instruments, Winooski, VT, USA)and 96-Well Plate were used for determination of spectral absorption values.

Antioxidant activity (Determination of 1,1-Diphenyl-2-picryhydrazyl (DPPH) radical-scavengingcapacity)

The Antioxidant activity of the plant extract was determined based on the radical scavenging ability in interacting with a stable DPPH free radical according to shimada et al. (1992)with some modifications. Briefly,1 mM DPPH in methanol was prepared and 50 µl of the solution was added to 150 µl of methanol plant extract (50- 300 µg/ml).This mixture was agitated vigorouslyand placed in darkness for 30 min at room temperature and then the absorbance was determined at 517 nm.The radical scavengingactivityof BHT was determined as the positive control. Thelower absorbance of reaction mixture indicates higher free radical scavenging activity. Purple colored stable free radicals are reduced to the yellow colored diphenylpicrylhydrazine when antioxidant was added.The following determination of the absorbance of the corresponding blank, DPPH radical scavengingactivity was determined usingthe following equation:

$$\%\ \text{inhibition of DPPH}' = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100$$

Where: Abs control =The absorbance of the control reaction (containing all reagents except thetested sample)

Abs sample =The absorbance in the presence of the tested extracts

The DPPHscavenging activity was expressed as IC_{50} value (the concentration of the antioxidant extract required to scavenge 50% of DPPH present in the tested solution).

In this assay, a microplate reader (Bio-Tek Instruments, Winooski, VT, USA) and 96-well plate were used for the determination of the spectral absorption values.

Statistical analysis

All analyses were carried out in triplicate samples (n=3) and expressed as mean±standard deviation (SD).Data generated were subjected to analysis of variance(ANOVA) and descriptive statistics, using statistical analysissystem (SAS) software package 9.2 (SAS Institute2000). Least significant difference (LSD) test was used formean comparison.

RESULTS AND DISCUSSION

Extractive yield

The extraction of bioactive compounds oflettuce outer leaves wascarried out for the valorization of this agricultural waste/by-product. In this study, three different extraction methods (UAE, MA and SE) were applied to extract the antioxidantsfromlettuce by-products. The yields of the obtained extracts are shown in Table1. The extractive yield (mass of extract/mass of dry matter) is considered as an indicator of the effects of the extraction conditions and as shownthe extraction techniques strongly affect the extractive yields that varied from 33 to 41 gextract/100 g of dry plant material for UAE (41%) and Maceration(33%), respectively.

Extractive yield and composition of the extracts are dependent on the extraction method and solvent polarity [32].Extraction technique is one of the main factors for achieving high quality antioxidant extract. It is clear that simple, fast and environment-friendly methodispreferred. Also the selected extraction technique needs to be non destructive showing high capacity for extraction of the most active effective matter. The efficient method should be capable of reducing extraction time and solvent consumption and improving extraction yield and extracts quality [42]. Ultrasound (18-40 KHz) is a green effective free-pollutantstechniquewhich facilitates the extraction process and reduces energy use and since ultrasound-assisted extraction might be carried out at low temperature it does not lead to extract degradation. Italsoprovides some advantages namely reproducibility, high efficiency and selectivity, short processing time, enhanced quality, reduced physical and chemical hazards and above all it is environmentally friendly [13]. Higher efficiency of ultrasonic extraction is due to cavitation effects.When the ultrasound waves pass through a liquid, micro bubbles are generated. Temperature and pressure inside these bubbles are high. When the microbubbles are collapsed violent shock wave and high speed jet are formed that promote the solvent penetration into cell wall and intracellular contents are released via cell wall decomposition [11].In UAE both cavitation and thermal effects are involved in the extraction process in contrast to the common methods where only the thermal effect is important for improving extraction yield [50].Different methods and solvents employed for extraction and their involvements depend on the stability and nature of phenolic compounds.

Polar solvents are applied in order to recover polyphenols from plant matrix [57].The most commonly applied solvents are aqueous methanol, ethanol and acetone mixtures [39, 52, 10].Nontoxic food grade and environment-friendly solvents such as ethanol, n-butanol and iso propanol are recommended for extraction purposes by U.S. Food and Drug Administration [9]. Ethanol and water mixtures are commonly used for the extraction of phenols from plant materials[8,17].The reason is the solubility of a wide range of phenols in aqueous ethanol mixture that is non-toxic and food grade[3].Therefore in this study EtOH/H_2O(70:30, v/v), was selected. The results showed that ultrasound-assisted extraction significantly increased the extraction yield of lettuceby-products extract within a shorter time and is in consistent with the findings reported by other researchers [42, 6, 61, 47, 50].Rathod S. and Rathod V. (2014) compared the ultrasound-assisted extraction of Piperine from Piper longumwith conventional extraction technique.They showed that the extraction time is reduced from 8 h of batch solvent extraction and 4 h of Soxhletto 18 min in UAE with enhanced extraction yield of piperine.

Tabaraki and Rastgoo (2014)investigated the conventional and ultrasound-assisted extraction methods of natural antioxidants from walnut green husk. They stated that the yield obtained by UAE during 30 min (37.52%) was

significantly higher than the conventional extraction method(14.31%).Rezaie et al. (2015) compared the ultrasound-assisted extraction (10, 30, and 60 min at 0°C) of antioxidative compounds from Bene (Pistacia atlantica subsp. mutica) hull with conventional extraction technique (24 h at room temperature). They showed thatthe yields of extracts were significantly improved by using the ultrasound and the best results wasobserved by using a 30min sonication process.

Mostof the studies carried outon theantioxidative extracts oflettuceleaves have been concerned with the application of conventionalmethod of extraction.There are not reportsconcerned with the application of ultrasound-assisted extraction of antioxidant compoundsfromlettuce by-products. In this research work the extractive yield was much higher as compared to other research works carried out using conventional method of extraction.Llorach et al. (2004) evaluated lettuce and chicory by-products as a source of antioxidant extracts.They investigated the extraction yield, phenolic content and antioxidant capacity of the extracts obtained by refluxing the by-products with the boiling solvent (1:3 w/v) (methanol or water) for 1 h. Baby and romaine lettuce by-products showed the highest water extract yields (27 and 26 g of freeze-dried extracts/kg of by-product FW, respectively), whereas baby and iceberg lettuce showed the highest methanol extractive yields (31 and 23 g of freeze-dried extracts/kg of by-product FW, respectively).

Total phenolic contents

Total phenolic contents (TPC) were determined using Folin-Ciocalteu reagent. Folin-Ciocalteu reagent reacts nonspecifically with phenolic compounds as it might be reduced by some of non phenolic compounds (e.g., Ascorbic acid and Cu II).The exact reaction of the reagent and reducing species is unknown, however in the reaction of phosphomolybdic tungstate and reducing species, phenolate ion, a complex is formed with the color being changed from yellow to blue and the absorbance is determined at 760 nm against Gallic acid as the standard [27].

The results concerned with the TPC in the lettuce extracts are shown in Table 1.The TPC varied from 474.72to 791.10 mg Gallic acid equivalents/100g of dry matterdepending on the applied extraction technique.

Statistically significant differences ($p < 0.05$) were between TPC of extractsusing different extraction techniques.The highest and lowest TPC of extracts were observed obtained by UAE (791.10mg Gallic acid equivalents/100g Dry Weight) and Maceration (474.72 mg Gallic acid equivalents/100g DW), respectively.The results of this study indicated the efficiency of ultrasound waves for extraction of the phenolic contentsthat are in agreement with the results reported by other researchers[42, 6, 47, 61, 51]. Nantitanon et al. (2010) investigated the influence of certain factors on the extractive yields, antioxidant activitiesand total phenolic contentsof guava leaf extracts. Theydemonstrated that the ultrasonication technique resulted in the extract with the highest TPC whereas the maceration without stirring yielded the extract with the lowest TPC.

TabarakiandRastgoo (2014)showed that TPC of extracts of walnut green husk obtained by UAE was significantly higher (6.83mg Gallic acid equivalents/g DW) than theconventional extraction (4.25 Gallic acid equivalents/g DW).Amirah et al. (2012) applied different extraction techniques for obtaining Gallic acid from Jatropha curcas stem bark. They concluded that Gallic acid was degraded easily when exposed to high temperatures for long periods of time in soxhlet extraction method.

Szydłowska-Czerniak and Tułodziecka (2014) applied ultrasound-assisted extraction and conventional solid–liquid extraction to obtain antioxidant extracts from two rapeseed varieties. They stated that TPC in rapeseed extractsobtained by the UAE during 18 minwas significantly higher than by the conventional extraction for 90 min.Rezaie et al. (2015) showed that TPC of extracts of Bene (Pistacia atlantica subsp. mutica) hull obtained by UAE (30min at 0°C) was significantly higher than by conventional extraction (24 h at room temperature).

Our findings concerned with the application of the ultrasound to lettuce by-products indicated high quantities of phenolic antioxidants extracts were isolated as compared to other researchers who employed the conventional method of extraction.Gomes et al. (2013)determined the TPC of lettuce extract obtained 0.335 mg Gallic acid equivalents/g Fresh weightusing 60% (v/v) methanol/water and0.378 mg Gallic acid equivalents/g Fresh weight using acetone/waterunder stirring extraction at 45°C and 35 min.Ozgen andSekerci (2011) examined lettuce outer leaves of Krizet and Freckles varieties (bothgreen cultivars) and obtained 0.214–0.431 mg Gallic acid equivalents/g Fresh weight usingacetone, water and acetic acid at ratios of 70:29.5:0.5, v/v at 4°C for 24 h.Llorach et al. (2008) investigated the characterisation of polyphenols and the antioxidant activities offive lettuce varieties using mixtures of MeOH/water/formic acid (25/24/3,v:v:v) and found thetotal phenolic contentof Romaine lettucein order of 63.5±3.5 (mg Gallic acid equivalents/100 g Fresh weight).L´opez et al. (2014) evaluated the chemical composition and antioxidant capacity of lettuce (Romaine,Little Gem and Mini Romaine) types. Romaine type showed the highest content of phenolic compounds.

The differences might be due to different extraction conditions, technique applied and solvent type used by these authors. According to Gobbo-Neto and Lopes (2007) different factors including season, temperature, water availability, UV radiation, soil quality, pollutionand diseases mightinfluence the secondary metabolites contents such as phenolic contents in the plants.

Antioxidant activity

1,1-Diphenyl-2-Picryllydrazyl (DPPH) is a stable organic radical nitrogen. DPPH radical is commercially available. Evaluation of DPPH scavenging is widely used for determination of the antioxidant capacity of plant extracts. DPPH inhibition percentage indicates the antioxidant capacity of the analyzed extract. The time of measurement varies from 10-20 min to 6 h [23].

IC_{50} is mostly used to express the concentration of the extract required for scavenging 50% of free radicals. IC_{50} is conversely proportional to the antioxidant activity of plant extract.Free radical DPPH inhibition percentage at different concentrationsof ethanol extracts obtainedemploying maceration, ultrasonication and soxhletare shown in Fig. 1,2, 3.It is clear that at all the concentrations the antioxidant activity increases as the concentration increases. At 300 ppm concentrations, 70% (v/v)ethanol extracts of lettuce by-productsexhibited58.16% (soxhlet), 67.42%(maceration) and 76.59% (ultrasonication) antioxidant activities.The IC_{50} of the obtainedlettuce by-products extracts by three methods is shown in Fig.4. IC_{50} of the extract obtained by maceration was (228.60µg/ml), soxhlet (228.78µg/ml) and ultrasonication (192.22µg/ml).

The results of the present study suggests that the effectiveness of ultrasound technique in increasing the efficiency of the antioxidants extraction from plant matrix is consistent with the result obtained by other researchers[42, 61, 47, 51].Nantitanon et al. (2010) demonstrated and indicated that the ultrasonication technique has resulted inthe highest antioxidantactivities whereas the maceration without stirring obtained extract with the lowest antioxidantcapacities.Tabaraki andRastgoo (2014)showed theantioxidant activityof walnut green husk extract that was obtained by UAEduring30min that was significantly higher(34.21%) thanthe conventional extraction during 16 hours (23.55 %).Szydłowska-Czerniak and Tułodziecka(2014) reported thatthe application of ultrasound allowed the highest antioxidant activitiesas compared tothe conventional extraction.Rezaie et al. (2015) showed that the antioxidant activity of the extracts from Bene (Pistacia atlantica subsp. mutica) hull is (24 h at room temperature) significantly improved using ultrasonic waves.

Our finding regarding the antioxidant activities of the extracts in this study concerned with the application of ultrasonic waves agrees to some extent with the findings by other researchers who studied the conventional methods of extraction[35,25].Therefore ultrasonication might be employed as a new and effective method that consumes less

amounts of solvent, energy and time and above allit is environment friendly[48].Gomes et al.(2013) determined the DPPH scavenging effect of lettuce extract obtained with 60% (v/v) methanol/water (87.1%) and acetone/water (88.35%) under stirring extraction at 45°C and 35 min.Liu et al. (2007) evaluated the total phenolic contents and antioxidant activities of 25 cultivars of lettuce (Lactuca sativa L.) grown in Colorado. They determined the antioxidant activity of Romaine lettuce extract using acetone-water (1:1, v/v) at the ambient temperature for 15 h maceration in the order of 70%.

The evidence suggests that genotype and growing conditions mightalter the composition and antioxidant properties of agriculturalcrops[35].The variations in the biological activities of extracts obtained by different extraction methods have been also reported. Therefore selection of a proper extraction technique as well as solvent based on the matrix characteristics, chemical properties of analytes, matrix analyte interaction and efficiency is quite important[28, 29].

Influencesof solvent to solid ratios

The influences of solvent to solid ratioson the extractiveyields, TPC and antioxidant activities of extracts obtainedbythe ultrasound-assisted extractionis shown at Table2 and Fig.5, respectively.

A marked increase in the yieldof the extract up to 29%was observed using 20:1(v/w)solvent to solid ratio followed by a slight increase using80:1(v/w) solvent to solid ratio (Table 2).

This is consistent with mass transfer principles; the driving force during mass transfer is the concentration gradient between the solid and the solvent and it is greater when a higher solvent to solid ratio is used[2].However, higher solvent to solid ratio may mean more solvent usage in extraction and energy consumption for concentration in the next step of processing.

Rathod S. and RathodV.(2014) applied different solid to solvent ratios (1:2.5, 1:5, 1:10, 1:20, 1:30, 1:40 g/ml)in ultrasound-assisted extraction of piperine from Piper longum. Theyreported that the amount of extractedpiperine is increased with the decreasein solid to solvent ratio from 1:2.5 till 1:10. As the solute concentration wasincreased, the solvent slowly became saturated.

Tabaraki et al.(2012) investigated the effect of solvent to solid ratio on the ultrasonic-assisted extraction of pomegranate peel antioxidants with five ratios (20:1, 30:1, 40:1, 50:1 and 60:1; v:w) over a 20 min extraction period, with a 50% ethanol solution, at 45°C.Their study showed that the yield was increased with the increase in the solvent to solid ratio, however, the difference between50:1 and 60:1 solvent to solid ratioson the extraction yield was not significant.

The results showed that TPCwere increased with the increase in the solvent to solid ratio(Table 2).Extraction of phenolic compounds is a function of solid to liquid ratio. The solvent to solid ratio has a positive effect as the higher the solvent to solid ratio will resultin higher phenolicyieldsand greater volume of solvent isolates higher bioactive compounds[65, 66], scince it might facilitate the diffusion of the compounds thereby increasedphenolic compoundsis obtained[34]. Our results agree with those reportedby Sun et al. (2011), who confirmed an increase of yield inphenolic compounds when the solvent to solid ratio was increased from5:1 to 15:1 ml/g.MuñizMarquez et al.(2013) investigated the ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L.and their antioxidant activity. They found that by decreasing the solid to solvent ratio from 1:4 to 1:12g/mlthe yield of phenolic compoundswas increased.D'Alessandro et al. (2012) evaluated the influence of solid to solvent ratio on the ultrasound-assisted extraction of polyphenols from Aronia melanocarpa berries. They foundthat the concentration of phenolic compounds grows logically while the ratio increases. The yield of polyphenols at the ratio of 1:10 (44.0%) was sensibly lower than the extractive yields at ratios of 1:20 (57.5%) and 1:40 (56.9%) that were quite similar.

Fig.5 indicates thatthe highest antioxidant activity of the extract was at 78.61%, when the solvent to solid ratio was at the 20:1.This is probablydue to the greater chance of antioxidants to be in contact with the solvent as the solvent quantity is increased, however more increasing the solventwill result in reduced antioxidant activity[49].Therefore, it is suggestedthat solvent to solid ratio of 20:1 was sufficient forextraction of antioxidant extract from the lettuce by-products. Saturation of the antioxidantcompounds within the extraction solvent caused the highantioxidant properties.

Gan and Latiff (2011) reported high antioxidant activity when using solvent to solid ratio of 20ml/g for optimization of solvent extraction of bioactive compounds from parkia speciosa pod (at ratios of 20:50 ml/g).Prasad et al. (2011) investigated the influence of liquid-to-solidratio (20–50 ml/g) on the recovery of total phenolic contents and the antioxidant activities of Mangifera pajang peel. Their study showed the highest antioxidant activity with low ratio ofsolvent to solid (20–30 ml/g).

CONCLUSION

Lettuce wastes and by-products might serve asan inexpensive, natural antioxidants for the protection of functional and formulated food[37].The recovery of bioactive compounds from lettuce wastes and by-products might be an effective strategy to manage andcontrol the environmental pollution in this respect.Currently ultrasound-assisted extraction has been successfully usedto extraction thebioactivecompounds[59].Thus different extraction techniques consisting of maceration, ultrasound-assisted extraction and soxhlethave been investigated for the extraction of antioxidants from outer leaves of lettuce andthe methods arecompared based on the extraction yield, total phenolic contents and antioxidant activities. The results showed that extraction techniques and solvent to solid ratio strongly affected the above parameters ($p < 0.05$). It was also found that UAE significantly reduced the time of extraction and increased the extract yield, total phenolic contents as well as the antioxidant activity of lettuce wastes and by-products as compared to the conventional extraction methods. This technique has been accepted as an effective alternative method for extraction of natural phytoconstituents. Therefore ultrasound-assisted extraction of lettuce by-products is a green process yielding an isolate that is valuable, natural and is a good source of antioxidants.

REFERENCES

1. Agostini, F., Bertussi, R.A., Agostini, G., Atti dos Santos, A.C., Rossato, M., Vanderlinde, M. (2012). Supercritical extraction from vinification residues: fatty acids, atocopherol, and phenolic compounds in the oil seeds from different varieties of grape. Scient. World J.http://dx.doi.org/10.1100/2012/790486.

2. Al-Farsi, M.A.,Lee, C.Y. (2008). Optimization of phenolics and dietary fibre extraction from date seeds. Food Chem. 108, 977–985.

3. Alothman, M., Bhat, R., Karim, A.A.(2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry, 115, 785–788.

4. Altunkaya, A., Becker, E.M., Gökmen, V., Skibsted, L.H. (2009). Antioxidant activity of lettuce extract (Lactuca sativa) and synergism with added phenolic antioxidants. Food Chem. 115, 163–168.

5.Altunkaya, A., Gökmen, V.(2008). Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (Lactuca sativa). Food Chem. 107, 1173–1179.

6. Amirah, D.M., Reddy, P., Maksudur, R.K.(2012).Comparison of extraction techniques on extraction of Gallic acid from stem bark of Jatropha curcas, J. Appl. Sci. 12,1106–1111.

7. Andre, C. M., Larondelle, Y., Evers, D. (2010). Dietary antioxidants and oxidative stress from a human and plant perspective: A review. Current Nutrition & Food Science, 6, 2–12.

8. Bahorun, T., Luximon-Ramma, A., Crozier, A., Aruoma, O. I. (2004). Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. Journal of the Science of Food and Agriculture, 84,12, 1553–1561.

9. Bartnik, D.D., Mohler, C.M. & Houlihan M.(2006).Methods for the production of food grade extracts, United States Patent Application, 20060088627, April 27.
10. Borges, G. D. S. C., Vieira, F. G. K., Copetti, C., Gonzaga, L. V., Fett, R. (2011). Optimization of the extraction of flavanols and anthocyanins from the fruit pulp of Euterpeedulis using the response surface methodology. Food Research International, 44, 708–715.
11. Briars,R.,Paniwnyk, L. (2013). Effect of ultrasound on the extraction of artemisinin from Artemisia annua Ind. Crop. Prod. 42, 595–600.
12. Chandrapala, J., Oliver, C.M., Kentish, S., Ashokkumar, M.(2013). Use of power ultrasound to improve extraction and modify phase transitions in food processing, Food Rev. Int. 29 67–91.
13. Chemat, F., Huma, Z., Muhammed, K.K.(2011). Ultrason. Sonochem. 18,813–835.
14. Coletta, A., Trani, A., Faccia, M., Punzi, R., Di palmo, T., Crupi, P., Antonacci, D.,Gambacorta, G. (2013).Influence of viticultural practices and winemaking technologies on phenolic composition and sensory characteristics on Negramaro red wines, Int. J. Food Sci. Technol. 48, 2215–2227.
15. D'Alessandro, L.G., Kriaa, K., Nikov,I., Dimitrov, K.(2012).Ultrasound-assisted extraction of polyphenols from black chokeberry. Separation and Purification Technology, 93, 42–47.
16.Dupont, S., Mondi, Z., Willamson, G., Price, K. (2000). Effect of variety, processing, and storage on the flavonoid glycoside and composition of lettuce and chicory. Journal of Agricultural and Food Chemistry, 48, 3957–3964.
17. Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y.,Perry,N. B.(2007). Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using ethanol–water mixtures. Food Chemistry, 101, 1417–1424.
18. Feng, S., Luo, Z., Tao, B. Chen, C. (2015).Ultrasonic-assisted extraction and purification of phenolic compounds from sugarcane (Saccharum officinarum L.) rinds. LWT - Food Science and Technology, 60,970–976.
19. Ferreira, A. (2011). Influence of spent coffee grounds on growth and chemical and biological properties of lettuce (Lactuca sativa L.). Master Thesis. School of Agriculture, Polytechnic Institute of Braganc¸ a, Portugal.
20. Gan, C., Latiff, A. A. (2011). Optimisation of the solvent extraction of bioactive compounds from Parkia speciosa pod using response surface methodology. Food Chemistry, 124, 1277–1283.
21.Giovannini, C., Masella, R. (2012). Role of polyphenols in cell death control. Nutritional Neuroscience, 15, 134–149.
22. Ghitescu, R.E., Volf, I., Carausu, C., Bühlmann, A.M., Gilca, I.A., Popa, V.I. (2015). Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark. Ultrasonics Sonochemistry, 22, 535–541.
23. Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holeroft, D. M., Kader, A. A.(2000). Antioxidant activity of pomegranate juice and its relationship withphenolic composition and processing. Journal of Agriculture and Food Chemistry,48, 4581–4589.
24. Gobbo-Neto, L., Lopes, N.P. (2007). Plantas medicinais: Fatores de influência no conteúdo demetabólitos secundários. Quim. Nova, 30, 374–381.
25.Gomes,T., Delgado,T., Ferreira,A., Pereira, J.A.,Baptista,P., Casal,S.,Ramalhosa,E.(2013). Application of response surface methodology for obtaining lettuce. (Lactuca sativa L.) by-products extracts with high antioxidative properties. J. Industrial Crops and Products, 44, 622– 629.
26. Gong, Y., Hou, Z., Gao, Y., Xue, Y., Liu, X., Liu, G. (2012).Optimization of extraction parameters of bioactive components from defatted marigold (Tagetes erecta L.) residue using response surface methodology, Food Bioprod. Process, 90, 9–16.
27.Haung, D.; Ou, B.; Prior, R. L. (2005).The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry,53, 1841–1856.
28.Hayouni, E.A., Abedrabba, M., Bouix, M., Hamdi, M.(2007). The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian Quercus coccifera L. and Juniperus phoenica L. fruit extracts. Food Chem. 105, 1126–1134.
29. Ishida, B.K., Ma, J., Bock, C.(2001). A simple rapid method for HPLC analysis of lycopene isomers. Phytochem. Anal. 12, 194–198.
30. Kabir, F., Tow, W.W., Hamauzu, Y., Katayama,S., Tanaka, S., Nakamura,S. (2015).Antioxidant and cytoprotective activities of extracts prepared from fruitand vegetable wastes and by-products. Food Chemistry, 167, 358–362.

31. Leopoldini, M., Russo, N., Toscano, M. (2011). The molecular basis of workingmechanism of natural polyphenolic antioxidants. Food Chemistry, 125, 288–306.
32. Li, Y., Guo, C., Yang, J., Wei, J., Xu, J.,Cheng, S.(2006). Food Chem.96, 254–260.
33. Li, Z., Zhao, X., Sandhu, A.K., Gu, L.(2010). Effects of exogenous abscisic acid on yield, antioxidant capacities, and phytochemical contents of greenhouse grown lettuces. J. Agric. Food Chem. 58, 6503–6509.
34. Liu, Q.M., Yang, X.M., Zhang, L., Majetich, G.(2010). Optimization of ultrasonic-assisted extraction of chlorogenic acid from Folium eucommiae and evaluation of its antioxidant activity, J. Med. Plants Res. 23, 2503–2511.
35.Liu, X., Ardo, S., Bunning, M., Parry, J., Zhou, K.,Stushnoff, C., Stoniker, F, Yu, L., Kendall, P.(2007).Total phenolic content and DPPH radicalscavenging activity of lettuce (Lactuca sativa L.)grown in Colorado. LWT 40, 552-557.
36. Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F.A., Gil, M.I., Ferreres, F. (2008).Characterisation of polyphenols and antioxidant properties of five lettuce varietiesand escarole. Food Chem. 108, 1028–1038.
37. Llorach, R., Tomás-Barberán, F.A., Ferreres, F. (2004). Lettuce and chicory by-products as a source of antioxidant phenolic extracts. J. Agric. Food Chem. 52, 5109–5116.
38. L´opez, A., Javier, G.A., Fenoll, J., Hell´ın,P., Flores, P. (2014).Chemical composition and antioxidant capacity of lettuce: Comparative study of regular-sized (Romaine) and baby-sized (Little Gem and Mini Romaine) types.Journal of Food Composition and Analysis,33, 39–48.
39. Ma, Y. Q., Chen, J. C., Liu, D. H., Ye, X. Q. (2009). Simultaneous extraction of phenoliccompounds of citrus peel extracts: Effect of ultrasound. Ultrasonics Sonochemistry, 16, 57–62.
40. Mulabagal et al. (2010). In vitro evaluation of red and green lettuce (Lactuca sativa) for functional food properties. Food Chemistry, 118, 300–306.
41. Muñiz-Márquez,D.B.,Martínez-Ávila, G.C., Wong-Paz, J.E., Belmares-Cerda, R., Rodríguez-Herrera, R., Aguilar, C.N.(2013).Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity, Ultrason. Sonochem. 20,1149–1154.
42. Nantitanon, W., Yotsawimonwat, S., Okonogi, S. (2010). Factors influencing antioxidant activities and total phenolic content of guava leaf extract. LWT - Food Science and Technology, 43, 1095-1103.
43. Nicolle, C., Cardinault, N., Gueux, E., Jaffrelo, L., Rock, E., Mazur, A., Amouroux, P., Rémésy, C.(2004). Health effect of vegetable-based diet: lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. Clin. Nutr. 23, 605–614.
44. Ozgen, S., Sekerci, S. (2011). Effect of leaf position on the distribution of phytochemicals and antioxidant capacity among green and red lettuce cultivars. Span. J. Agric. Res. 9, 801–809.
45. Pan, G., Yu, G., Zhu, C., Qiao, J.(2012). Optimization of ultrasound-assisted extraction (UAE) of flavonoids compounds (FC) from hawthorn seed (HS), Ultrason. Sonochem. 19, 486–490.
46. Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., García, I., Jiménez, D.,Lamuela- Raventós, R., Buxaderas S. ,Codina C. (2006). An industrial approach in the search of natural antioxidants fromvegetable and fruit wastes. Food Chemistry, 97, 137–150.
47. Porto, C.D., Porretto, E. , Decorti, D. (2013). Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (Vitis vinifera L.) seeds. Ultrasonics Sonochemistry, 20, 1076–1080.
48. Prakash Maran, J., Manikandan, S., Vigna Nivetha, C., Dinesh, R. (2013).Ultrasound-assisted extraction of bioactive compoundsfrom Nephelium lappaceum L. fruit peel using centralcomposite face centered response surface design.Arabian Journal of Chemistry,http://dx.doi.org/10/1016/j.arabjc.2013.002.007.
49. Prasad, K. N., Hassan, F. A., Yang, B., Kong, K. W., Ramanan, R. N.,Azlan, A., & Ismail, A. (2011). Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of underutilised Mangifera pajang Kosterm. Peels. J. Food Chemistry, 128, 1121–1127.
50. Rathod, S.S., Rathod, V.K. (2014). Extraction of piperine from Piper longum using ultrasound. Industrial Crops and Products, 58,59–264.
51.Rezaie, M., Farhoosh, R., Iranshahi, M., Sharif, A., Golmohamadzadeh, Sh.(2015).Ultrasonic-assisted extraction of antioxidative compounds from Bene (Pistacia atlantica subsp. mutica) hull using various solvents of different physicochemical properties. Food Chemistry, 173, 577–583.

52. Rodrigues, S., Pinto, G. A. S., Fernandes, F. A. N.(2008).Optimization of ultrasoundextraction of phenolic compounds from coconut (Cocos nucifera) shell powder byresponse surface methodology. Ultrasonics Sonochemistry, 15, 95–100.

53. Serafini, M., Bugianesi, R., Salucci, M., Azzini, E., Raguzzini, A., Maiani, G. (2002). Effect of acute ingestion of fresh and stored lettuce (Lactuca sativa) on plasma total antioxidant capacity and antioxidant levels in human subjects. Br. J. Nutr. 88, 615–623.

54.Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T.(1992).Antioxidative properties ofxanthin on auto oxidation of soybean oil in cyclodextrin emulsion. Agricultural and FoodChemistry, 40, 945-948.

55.Shyamala, B.N., Gupta, S., Lakshmi, A.J., Prakash, J.(2005). Leafy vegetable extracts-antioxidant activity and effect on storage stability of heated oils. Innov.Food Sci. Emerg. T., 6, 239-245.

56. Singleton, V.L., Rossi, J.A.(1965).Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am. J. Enol. Vitic.16, 144-158.

57. Sultana, B., Anwar, F., Przybylski, R.(2007). Food Chem. 104,1106–1114.

58. Sun, Y., Liu, Z., Wang, J.(2011). Ultrasound-assisted extraction of five isoflavones from Iris tectorum Maxim, Sep. Purific. Technol. 78, 49–54.

59. Szydłowska-Czerniak, A., Tułodziecka, A. (2014).Antioxidant capacity of rapeseed extracts obtained by conventional and ultrasound-assisted extraction.J Am Oil Chem Soc. 91,2011–2019.

60. Tabaraki, R., Heidarizadi, E., Benvidi, A. (2012). Optimization of ultrasonic-assisted extraction of pomegranate (Punica granatum L.) peel antioxidants by response surface methodology.Separation and Purification Technology, 98,16–23.

61. Tabaraki,R., Rastgoo, S.(2014).Comparison between conventional and ultrasound-assisted extractions of natural antioxidants from walnut green husk. Korean J. Chem. Eng., 31, 676-683.

62. Wang, L., Weller, C.L.(2006). Recent advances in extraction of nutraceuticals fromplants. Trends Food Sci. Technol. 17, 300–312.

63. Wijngaard, H. H., Rößle, C., Brunton, N. (2009). A survey of Irish fruit andvegetable waste and by-products as a source of polyphenolic antioxidants. FoodChemistry,116, 202–207.

64. Wong Paz, J.E., Muñiz Márquez, D.B., Martínez Ávila, G.C., Belmares Cerda, R.E., Aguilar, C.N. (2015). Ultrasound-assisted extraction of polyphenols from native plants in the Mexican desert. Ultrasonics Sonochemistry, 22, 474–481.

65.Xia, E.Q., Ai, X.X., Zang, S.Y., Guan, T.T., Xu, X.R., Li, H.B. (2011). Ultrasound-assisted extraction of phyllyrin from Forsythia suspensa, Ultrason. Sonochem. 18 ,549–552.

66. Olfati A, Moghaddam GH, Moradi Kor N, Bakhtiari M. 2014. The Relationship between Progesterone and Biochemical Constituents of Amniotic Fluid with Placenta Traits in Iranian Crossbred Ewes (Arkhar-Merino×Ghezel). Asian Pacific Journal of Tropical Medicine. 7 (Suppl 1): S162-S166.

Table 1.The yield andthe total phenolic contents of the extracts obtained from different extraction methods

Extraction method	Yield (g/100g DW)	TPC (mg GAE /100g DW)
Maceration with stirring(200rpm)	33.00 ± 0.80[a]	474.72 ± 7.14[c]
Ultrasonication	41.00 ± 1.23[b]	791.10 ± 5.01[d]
Soxhlet	34.4 ± 1.60[a]	597.59 ± 4.16[e]

∗Dissimilar letters represent significant difference at p<0.05.

Table 2. The yield and the total phenolic contents of extracts with different Solvent to solid ratios

Solvent to solid ratio (V:W)	Yield (g/100g DW)	TPC (mg GAE/100DW)
10:1	20 ± 1.05[a]	278.40 ± 2.58[c]
20:1	29 ± 1.14[b]	428.57± 3.49[d]
40:1	30 ± 0.90[b]	471.77± 5.60[e]
80:1	30 ± 1.29[b]	507.21± 4.37[f]

∗Dissimilar letters represent significant difference at p<0.05.

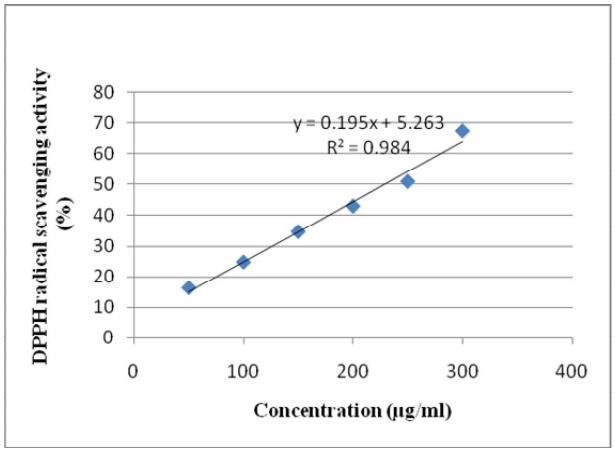

Fig.1. Free radical DPPH inhibition (%) at differentconcentrations ofethanol extractobtained by Maceration

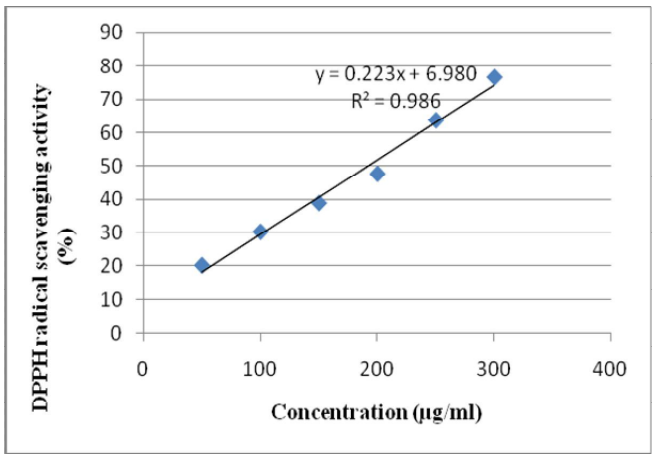

Fig.2. Free radical DPPH inhibition (%) at different concentrations of ethanol Extract obtained by ultrasonication

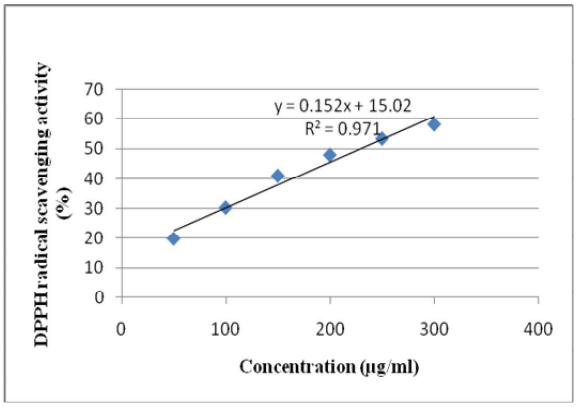

Fig.3. Free radical DPPH inhibition (%) at different concentrations of ethanol extract obtained by soxhlet

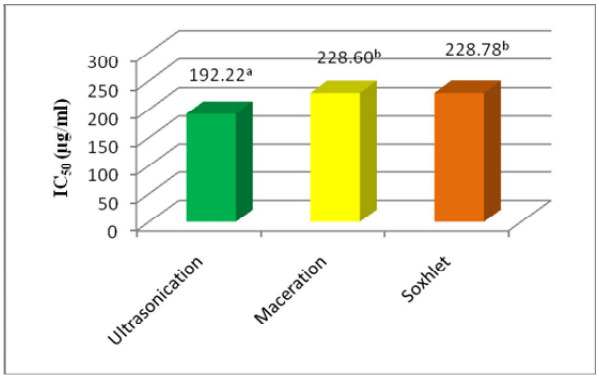

Fig.4. IC50 of ethanol extract obtainedby ultrasonication, maceration and soxhelt
∗ Dissimilar letters represent significant difference at p<0.05.

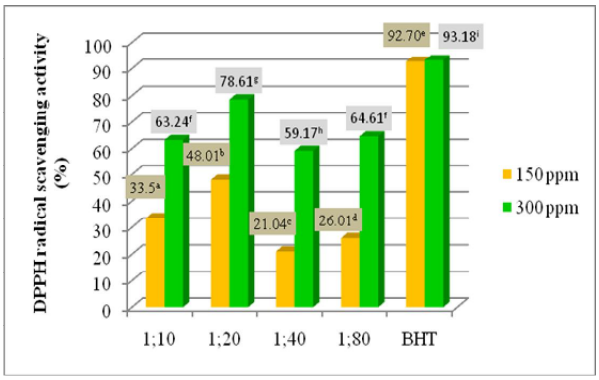

Fig.5. Free radical DPPH inhibition (%) at different concentrations of ethanol extract obtained by ultrasonication and BHT at different ratios.
∗ Dissimilar letters represent significant difference at p<0.05.

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Changes in Yield and Yield Components of Maize (*Zea mays* L.) under Different Methods of Application of N and P Bio-Fertilizers

Amin Farnia[1]* and Fereidoon Shahbazi[2]

[1]Assistant Professor of Department of Agriculture, Boroujerd Branch, Islamic Azad University, Boroujerd, Iran.
[2]M.Sc student of Department of Agriculture, Boroujerd Branch, Islamic Azad University, Boroujerd, Iran.

Received: 29 Mar 2015 Revised: 26 Apr 2015 Accepted: 26 May 2015

***Address for correspondence**
Amin Farnia
Assistant Professor of Department of Agriculture,
Boroujerd Branch, Islamic Azad University,
Boroujerd, Iran.
E-mail: Aminfarnia@yahoo.com.

ABSTRACT

This study was carried out in order to evaluate the effect of different methods of application of N and P bio-fertilizers on yield and yield components of maize (NS640 cultivar). This experiment was conducted in Islamic Azad University of Boroujerd, Iran, during the growing season of 2013- 2014. The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were different methods of application of Azot Barvar$_1$ as nitrogen bio-fertilizer (seed inoculation, top dressing, combined application of seed inoculation and top dressing) in main plots and different methods of application of phosphate Barvar$_2$ as phosphate bio-fertilizer (seed inoculation, top dressing, combined application of seed inoculation and top dressing) in sub plots and control treatment for them. Results showed that, the effect of nitrogen and phosphate application methods were significant on plant height, LAI, number of row per ear, number of grain per ear, biomass and grain yield. Interaction effect of them was significant on biomass only. Among the nitrogen application methods maximum of all traits was recorded at combined application of seed inoculation and top dressing treatment. However, among the phosphate application methods maximum of all traits was recorded at combined application of seed inoculation and top dressing treatment too. In final we find that combined application of seed inoculation and top dressing treatment for nitrogen and phosphate fertilizers was more useful for achieve to maximum yield and yield components of maize.

Key words: Application of fertilizer, seed inoculation and maize

INTRODUCTION

Corn (*Zea mays* L.) is one of the major cereal crops and is a very versatile grain that benefits mankind in many ways. It is a versatile crop and ranks third following wheat and rice in world production as reported by Food and Agriculture Organization (FAO, 2002). Corn or maize is one of the three most important cereal crops in the world. It is a versatile crop and ranks third following wheat and rice in world production as reported (FAO, 2002).

Conventional farming methods succeed in today's world is not acceptable to use the resource management and relying too much on synthetic inputs such as fertilizers and chemical pesticides can cause auxiliary power injector and the ecosystem of unstable farming (Roberts, 2008). The synthetic fertilizers are harmful for soil, because the inorganic fertilizers mainly contain major nutrients NPK in large quantities and are neglecting the use of organic manures and hence have paved the way for deterioration of soil health (Choudhry, 2005). Biofertilizers are microorganisms that help plants to grow by increasing the quantity of nutrients. Since these fertilizers contain living microorganisms, it increases or promotes the supply of important nutrients crucial for the overall productivity of the soil (Karthick et al, 2014). Many date application of fertilizers such as biofertilizers had positive effect on yield and quality of many crop plants rather than single application of them. Nitrogen fertilizers on the other hand, pose a health hazard and microbial population problem in soil besides the high cost of their application (Hasaneen et al., 2009) and application of it with P biofertilizer as integrated application of seed inoculation and top dressing produced high biomass yield rather than other treatments. El-Habbasha and Abd El-Salam (2010) illustrated that increasing nitrogen fertilization significantly increase bimass yield and decreased the oil content in canola seeds. Seed inoculation and the application of biofertilizers, products containing plant growth-promoting rhizobacteria have been used to reduce the negative effects of ethylene under stress conditions and increase of plant growth (Saleem et al., 2007) Moreover, some microorganisms like Azotobacter have multiple functions for plant growth which may derive both from their nitrogen fixation and stimulating effect on root development (Naderifar and Daneshian, 2012). Soil microorganisms, viz. Azotobacter and Azospirillum as N_2–fixing bacteria could be a beneficial source to enhance plant growth and produce considerable amounts of biologically active substances that can promote growth of reproductive organs and increase the plants' productivity (Yasari et al., 2009). Application of nitrogen and phosphate biofertilizers increased yield and yield components of barley under Boroujerd environmental condition (Azimi et al, 2013b). Nitrogen fertilizers on the other hand, pose a health hazard and microbial population problem in soil besides the high cost of their application (Hasaneen et al., 2009). El-Habbasha and Abd El-Salam (2010) illustrated that increasing nitrogen fertilization significantly increase bimass yield and decreased the oil content in canola seeds. Yasari and Patwardhan (2007) reported that application of Azotobacter and Azospirillum strains increased canola grain yield (21.17%), pod per plant (16.05%), number of branches (11.78%) and weight of 1000 grain (2.92%). These results are also in harmony with those obtained by Yasari et al. (2008, 2009).

Therefore this study was planned to examine effect of different methods of application of N and P bio-fertilizers in yield and yield components of maize (*zea mays* L.).

MATERIALS AND METHODS

This study was carried out in order to evaluate the effect of different methods of application of N and P bio-fertilizers in yield and yield components of maize (NS640 cultivar). This experiment was conducted in Islamic Azad University of Boroujerd, Iran (at Koohdasht region), during the growing seasons 2013- 2014. The experiment was laid out in a split-plot design based on randomized block design with three replications. Soil property of experimental field showed in table1.

Treatments were different methods of application of Azot Barvar$_1$ as nitrogen bio-fertilizer (seed inoculation, top dressing, combined application of seed inoculation and top dressing) in main plots and different methods of application of Phaphate Barvar$_2$ as Phsphate bio-fertilizer (seed inoculation, top dressing, combined application of seed inoculation and top dressing) in sub plots and control treatment for them.

In this field experiment there were 6 rows in each plots and rows were 6 m long with 0.75 m row spacing and plant to plant spacing was 18 cm too. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle 2m^2 of the four central rows were harvested. Then grain and biomass yield and yield components were calculated as standard methods with using 10 plants. Yield was defined in terms of grams per square meter and quintals per hectare. The harvest index was accounted for with the following formula:

HI = (economical yield / biological yield)

The statistical analyses to determine the individual and interactive effects of treatments were conducted using JMP 5.0.1.2 (SAS Institute Inc., 1997). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

RESULTS AND DISCUSSION

Plant height : The analysis of variance showed that, the effect of methods of application of N and P bio-fertilizers on plant height was significant only (table 2). The comparison of the mean values of the plant height for N bio-fertilizers showed that integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest (282cm) and control treatment had the lowest (268cm) plant height (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest (284cm) and control treatment had the lowest (265cm) plant height (table 4).

LAI : The effect of methods of application of N and P bio-fertilizers on LAI was significant only (table 2). The comparison of the mean values of the LAI for N bio-fertilizers showed that integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest (4.56) and control treatment had the lowest (3.71) LAI (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest (4.66) and control treatment had the lowest (3.69) LAI (table 4).

Number of row per ear :The analysis of variance showed that, the effect of methods of application of both N and P bio-fertilizers on number of row per ear was significant (table 2). For N bio-fertilizers, integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest (16.14) and control treatment had the lowest (14.69) number of row per ear (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest (16.43) and control treatment had the lowest (14.23) number of row per ear (table 4).

Number of grain per ear : The effect of methods of application of both N and P bio-fertilizers on number of grain per ear was significant (table 2). For N bio-fertilizers, integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest (640) and control treatment had the lowest (518) number of grain per ear (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest (641) and control treatment had the lowest (519) number of grain per ear (table 4).

Grain weight :The analysis of variance showed that, the effect of methods of application of P bio-fertilizers on 400grain weight was significant only (table 2). The comparison of the mean values of the plant height for P bio-fertilizers showed that integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest (126g) and control treatment had the lowest (114g) 400grain weight (table 4).

Biomass yield :The analysis of variance showed that, the effect of methods of application of N and P bio-fertilizers and interaction between them on biomass yield was significant (table 2). The comparison of the mean values of the biomass yield for N and P bio-fertilizers showed that combined integrated application of seed inoculation and top dressing of Azot Barvar$_1$ and Phosphate Barvar$_2$ had the highest (43 ton per ha) biomass yield (figure 1).

Grain yield: The effect of methods of application of N and P bio-fertilizers on grain yield was significant only (table 2). The comparison of the mean values of the grain yield for N bio-fertilizers showed that integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest (14.5 ton per ha) and control treatment had the lowest (13.4 ton per ha) grain yield (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest (15.5 ton per ha) and control treatment had the lowest (12.3 ton per ha) grain yield (table 4).

Harvest index :The any of treatments was not significant on harvest index (table 2).

The present study reviled that the effect of application methods of N biofertilizer were significant on all traits excepting grain weight and harvest index (table2). Also, the effects of application methods of P biofertilizer were significant on all traits excepting harvest index (table2). Interaction effect of N and P biofertilizers on biomass yield was significant only (table 2). Many researchers told that yield and yield components of many crops such as maize increased with application of biofertilizers as any application methods (Azimi et al, 2013a,b; Beyranvand et al, 2013). This may resulted from its ability to increase the availability of phosphorus and other nutrients especially under the specialty of the calcareous nature of the soil which cause decreasing on the nutrients availability. In the present study integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest and control treatment had the lowest plant height (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest and control treatment had the lowest plant height (table 4). The increased of plant height in integrated application of seed inoculation and top dressing of Azot Barvar$_1$ and Phosphate Barvar$_2$ may be attributed due to better plant development through efficient utilization of these biofertilizers by the plant in different application dates of them.

However, integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest and control treatment had the lowest LAI (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest and control treatment had the lowest LAI (table 4). Application of nitrogen and phosphate as integrated application of seed inoculation and top dressing had a positive effect of growth and development in maize and laid to the highest LAI in all treatment of application methods. In the present study for N bio-fertilizers, integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest and control treatment had the lowest number of row and grain per ear (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest and control treatment had the lowest number of row and grain per ear (table 4). Seed inoculation and the application of biofertilizers, products containing plant growth-promoting rhizobacteria have been used to reduce the negative effects of ethylene under stress conditions and increase of plant growth (Saleem et al., 2007) that this term laid to the production of highest number of row and grain per ear. Nabila et al. (2007) observed that application of Azospirillum as single on wheat had significant effect on number of grain per spikelet.

Biomass yield of maize was influenced significantly by application of biofertilizers.

The comparison of the mean values of the biomass yield for N and P bio-fertilizers showed that combined integrated application of seed inoculation and top dressing of Azot Barvar$_1$ and Phosphate Barvar$_2$ had the highest biomass yield (figure 1). This might be the result of the microorganisms can enhance plant growth by increasing the efficiency of biological fixation, enhancing the availability of trace elements and by the production of plant growth promoting substances (Gyanershwar et al, 1998). Reduction in biomass yield in control treatment may be due to nutritional imbalance and deficiency of certain important plant growth nutrients at various important growth stages like that of flowering, seed formation and seed maturity. Nitrogen fertilizers on the other hand, pose a health hazard and microbial population problem in soil besides the high cost of their application (Hasaneen et al., 2009) and application of it with P biofertilizer as integrated application of seed inoculation and top dressing produced high biomass yield rather than other treatments. El-Habbasha and Abd El-Salam (2010) illustrated that increasing nitrogen fertilization significantly increase bimass yield and decreased the oil content in canola seeds.

The comparison of the mean values of the grain yield for N bio-fertilizers showed that integrated application of seed inoculation and top dressing of Azot Barvar$_1$ had the highest and control treatment had the lowest grain yield (table 3). Also for P bio-fertilizers integrated application of seed inoculation and top dressing of Phosphate Barvar$_2$ had the highest and control treatment had the lowest grain weight and yield (table 4). The positive effect of integrated application of seed inoculation and top dressing N and P biofertilizers is due to synergistic effect of them on yield and growth of maize. Hamidi et al. (2007) reported that grain weight and grain yield increased by multiple inoculations compared to single inoculation. Kazemi et al. (2005) reported that soybean seed inoculation with rhizobial bacteria significantly increased seed thousand weight. Quah and Jafar (1994) found that the increase in seed weight of mungbean by the application of 50 kg N ha^{-1}. Asadi Rahmani et al. (2000) reported that during seed filling stage of soybean in treatments inoculated by B. japonicum bacteria, more photoassimilate transport to grain due to higher photosynthesis level and this factor can increase the seed size and seed weight.The increase in grain weight were derived mainly from increase in 100 seed weight and number of seeds per ear. This finding was supported by Yasari and Patwardhan (2007) who reported that application of Azotobacter and Azospirillum strains increased canola grain yield (21.17%), pod per plant (16.05%), number of branches (11.78%) and weight of 1000 grain (2.92%). These results are also in harmony with those obtained by Yasari et al. (2008, 2009). Ahmadi and Bahrani (2009) showed that single application of nitrogen fertilizer affected the oil content negatively and decreased it by 3.3% in canola but increased grain yield. Performance of biofertilizers could be explained by the fixation of sufficient atmospheric nitrogen, production of plant growth promoters, decreasing the ethylene production in plants and solubilization of minerals such as phosphorus (Karthikeyan et al., 2008 a, b). The decrease in grain weight in single application methods may be attributed due to deficiency of essential nutrients at the time of flowering and seed setting stage of plant, because at this stage phosphorous is required by the plant to complete such important growth stages like that of flowering and seed setting optimally, while N and P are required by the plant to improve seeds yield quality (Muhammad et al, 2006). Biofertilizer microorganisms are more suitable for high crop yield, protection from different pathogens and pesticides. They also help in maintaining soil health by decomposition of dead and decaying matters in the soils (Verma et al., 2010). In final we find that combined application of seed inoculation and top dressing treatment for nitrogen and phosphate fertilizers was more useful for achieve to maximum yield and yield components of maize.

REFERENCES

1. Ahmadi, M. and M. J. Bahrani .2009. 'Yield and yield components of rapeseed as influenced by water stress at different growth stages and nitrogen levels. AmEuras J Agric Environ Sci. 5 (6): 755-761.
2. Asadi Rahmani H, Saleh Rastin N, Sajadi A (2000). Investigate the possibility of predicting the need of inoculation of soybeen, based on the determine number of Bradyrhizobium japonicum bacteria and index of availability of soil nitrogen. Soil Water J. 12(7): 21-32.

3. Azimi SM, Farnia A, Shaban M, Lak M. 2013a. Effect of different biofertilizers on Seed yield of barley (Hurdeom vulgar L.), Bahman cultivar. International journal of Advanced Biological and Biomedical Research. Volume 1, Issue 5: 538-546.
4. Azimi SM, Nabati E, Shaban M Lak M. 2013b. Effect of N and P bio fertilizers on yield components of barley. International journal of Advanced Biological and Biomedical Research. Volume 2, Issue 2: 365-370.
5. Beyranvand, H., Farnia, A., Nakhjavan, S.H., & Shaban, M. (2013). Response of yield and yield components of maize (Zea maiz L.) to different bio fertilizers. International journal of Advanced Biological and Biomedical Research. 1 9; 1068-1077.
6. Choudhry, A.U., 2005. Higher-Value Organics, pp: 35–8. Pakistan and Gulf Economist.
7. El-Habbasha , S.F. and M.S. Abd El-Salam. 2010. Response of two canola varieties (Brassica napus L.) to nitrogen fertilizer levels and zinc foliar application. Int. J. Acad. Res., 2(2): 60-66.
8. FAO. 2002. Fertilizer and the future. IFA/FAO Agriculture Conference on Global food security and the role of Sustainability Fertilization. Rome, Italy. 16th-20th March, 2003, pp 1-2.
9. Gyanershwar, P., Nareshkumar, G. & Parckh, L.D. (1998). Effect of Buffering on the Phosphate Solubilizing Ability of Microorganisms. World J. Microbiol. Biotechnol., 14: 669-673.
10. Hasaneen,M. N. A., M. E. Younis and S. M. N. Toyrky. 2009. Plant growth, metabolism and adaptation in relation to strees conditions xxiii. salinitybiofertility interactive effects on growth, carbohydrates and photosynthetic efficiency of Lactuca sativa. Plant Omics J., 2: 60-69.
11. Karthick S. Raja Namasivayam, Subha Lakshmi Saikia and R.S.Arvind Bharani. 2014. Evaluation of Persistence and Plant Growth Promoting Effect of Bioencapsulated Formulation of Suitable Bacterial Biofertilizers. BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA,. Vol. 11(2), 407-415.
12. Karthikeyan, B., C. A. Jaleel, , G. M. A. Lakshmanan and M. Deiveekasundaram . 2008b . Studies on rhizosphere microbial diversity of some commercially important medicinal plants. Colloids Surf. B:Biointerf, 62: 143–145.
13. Karthikeyan, B., C. A. Jaleel, C. X. Zhao, M. M. Joe, J. Srimannarayan and M. Deiveekasundaram . 2008a. AM fungi and phosphorus levels enhances the biomass yield and ajmalicine production in Catharanthus roseus. Eur. Asia J. Biosci.2: 26–33.
14. Kazemi, S., Ghaleshi, S., Ghanbari, A. & Kianoush, G.E. (2005). Effects of planting date and seed inoculation by the bacteria on the yield and yield components of two soybean varieties. Agri. Sci. Nat. Resour. 12(4): 20-26.
15. MUHAMMAD NAEEM, JAVAID IQBAL, AND MUHAMMAD AHMAD ALIAS HAJI AHMAD BAKHSH. 2006. Comparative Study of Inorganic Fertilizers and Organic Manures on Yield and Yield Components of Mungbean (Vigna radiat L.). JOURNAL OF AGRICULTURE & SOCIAL SCIENCES. 2(4): 227–229.
16. Nabila, M., Zaki, M.S., Karima, M. & EL-Din, G. (2007). Growth and yield of some wheat cultivars irrigated with saline water in newly cultivated land as affected by biofertilization. Journal of Applied Science Researcg. 3:1121-1126.
17. Naderifar, M. and J. Daneshian. 2012. 'Effect of seed inoculation with Azotobacter and Azospirillum and different nitrogen levels on yield and yield components of canola (Brassica napus L.)'. Iranian Journal of Plant Physiology 3 (1), 619 - 626.
18. Quah, S. C. and Jafar, N. 1994. Effect of nitrogen fertilizer on seed protein of mungbean. Applied Biology Beyond the Year 2000 3rd year Malaysian Soc. Appl. Biol. 13-18 March, 1994, Kebansaan, Malaysia. pp:72- 74.
19. Roberts, T.L. (2008). Improving nutrient use efficiency. Turk J. Agric. 32:177-182.
20. Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol. 34: 635–648.
21. SAS Institute. Inc. 1997. SAS/STAT Users Guide. Version 6.12. SAS Institute Inc. Cary. NC.
22. Verma, J.P., J .Yadav and K. N. Tiwari. 2010. Application of Rhizobium sp. BHURC01 and plant growth promoting rhizobactria on nodulation, plant biomass and yields of Chickpea (Cicer arietinum L.). Int. J. Agric. Res. 5: 148- 156.
23. Yasari , E. , A. Esmaeli, A. M. Pirdashti and S. Mozafari . 2008. Azotobacter and Azospirillum inoculants as biofertilizers in canola (Brassica napus L.) cultivation. Asian J. Plant Sci. 7(5): 490-494.

24. Yasari, E. and A. M. Patwardhan. 2007. Effects of aztobacter and azospirillium inoculations and chemical fertilizers on growth and productivity of canola. Asi. J. Plant. Sci., 6(1):77-82.
25. Yasari, E., A . Esmaeli , M. A. E. Mozafari and M. R. Alashti. 2009. Enhancement of growth and nutrient uptake of rapeseed (Brassica napus L.) by applying mineral nutrients and biofertilizers. Pak. J. Bio. Sci. 12(2): 127- 133.

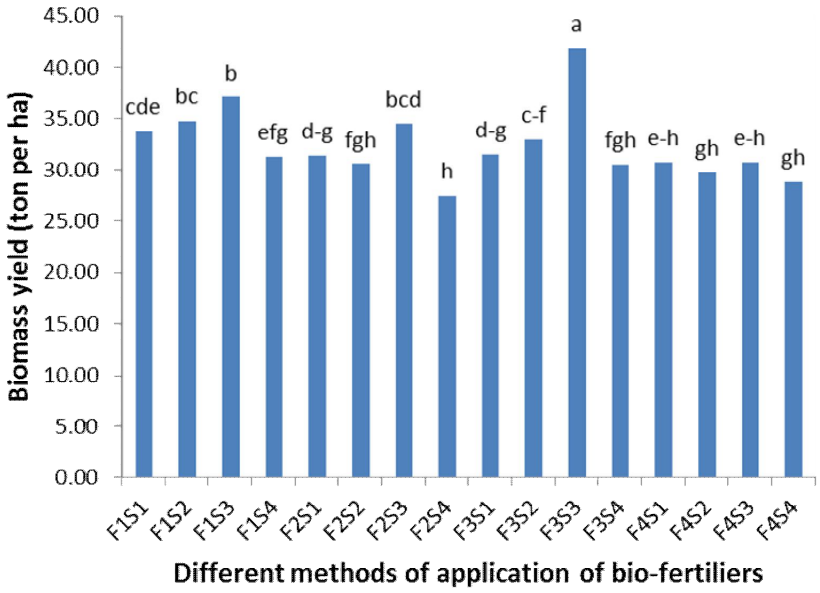

Figure 1. Effect of different methods of application of N and P bio-fertilizers on biomass yield of maize .

Means by the uncommon letter in each column are significantly different ($p<0.05$)

Different methods of application of Azot Barvar$_1$(F_1=seed inoculation(A), F_2=top dressing(B), F_3=integrated A and B and F_4=control)

Different methods of application of Phosphate Barvar$_2$(S_1=seed inoculation(A), S_2=top dressing(B), S_3=integrated A and B and S_4=control)

Table 1. Soil property of experiment site.

soil Texture	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	K (mg/kg)	P (ppm)	N (%)	pH	EC (ds/m)	Depth
Loam	5.2	0.68	4	0.32	275	4	0.2	7.7	1.39	0-60

Table2. Analysis of variance (mean squares) for yield and yield components of maize cultivars under different methods of application of N and P bio-fertilizers

S.O.V	df	Plant height	LAI	Number of row per ear	Number of grain per ear	grain weight	Biomass yield	Grain yield	Harvest index
R	2	98.66	0.11	0.06	2724.52	14.08	4.47	0.63	24.5
N application methods(A)	3	451.34*	1.81**	5.43**	41195.17**	369.8	57.85*	3.05*	46.2
Ea	6	61.72	0.08	0.29	2001.1	113.53	6.74	0.32	17.3
P application methods(B)	3	711.21**	1.88**	9.99**	19958.06**	334.69*	88.20**	21.51**	16.1
A x B	9	51.73	0.1	0.32	2257.56	59.41	12.88**	1.19	13.4
Eb	24	79.26	0.14	0.41	2701.01	43.42	2.8	0.91	7.8
Total	47	136.66	0.34	1.3	6086.33	93.61	14.27	2.33	22.2
CV(%)		2.85	6.97	3.53	7.74	8.83	8.02	4.08	6.5

ns: Non-significant, * and **: Significant at 5% and 1% probability levels, respectively

Table3. Simple means comparison for yield and yield components of maize cultivars under different methods of application of N bio-fertilizers

Methods of application of Azot Barvar$_1$	Plant height (cm)	LAI	Number of row per ear	Number of grain per ear	Grain Weight(g)	Biomass Yield (ton per ha)	Grain yield (ton per ha)	Harvest index (%)
Seed inoculation(A)	279 ab	4.40 a	15.69a	613.3a	118.3b	34.23a	13.74b	40.19b
Top dressing(B)	272 bc	3.99b	14.95b	538b	119.1b	30.98b	13.53b	43.67a
Integrated A and b	282.13 a	4.56a	16.14a	640.6a	128.8a	34.22a	14.52a	42.64a
Control	268.83 c	3.71c	14.69b	518.5b	116.3b	29.99b	13.40b	44.79a

Means by the uncommon letter in each column are significantly different (p<0.05)

Table4. Simple means comparison for yield and yield components of maize cultivars under different methods of application of P bio-fertilizers

Methods of application of Phosphate Barvar$_2$	Plant height (cm)	LAI	Number of row per ear	Number of grain per ear	Grain Weight(g)	Biomass Yield (ton per ha)	Grain yield (ton per ha)	Harvest index (%)
Seed inoculation(A)	276.875b	4.18b	15.61b	613.25a	119.08bc	31.85b	14.02b	44.22a
Top dressing(B)	274.625b	4.13b	15.20b	538b	122.08ab	32.02b	13.30b	41.74a
Integrated A and b	284.5833a	4.66a	16.43a	641a	126.92a	36.04a	15.52a	43.33a
Control	265.875c	3.69c	14.23c	519b	114.33c	29.51c	12.35c	42a

Means by the uncommon letter in each column are significantly different ($p<0.05$)

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.5 / Issue 30 / June 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Investigation of the role of GSTM1, GSTT1 and GSTP1 Polymorphisms in Chronic Obstructive Pulmonary Disease in a Smoker Population

TaherehAlahverdi[1], Sayyed-Mehdi Hashemi[2], LeylaPourgholi[1], Ali Mandegary[1,3]*

[1]Department of Pharmacology & Toxicology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.
[2]Department of Internal Medicine, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran.
[3]Gastroenterology and Hepatology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran.

Received: 17 Mar 2015 Revised: 25 Apr 2015 Accepted: 26 May 2015

***Address for correspondence**
Ali Mandegary
Department of Pharmacology & Toxicology,
School of Pharmacy, Kerman University of Medical Sciences,
Kerman, Iran.
E-mail: alimandegary@kmu.ac.ir, alimandegary@yahoo.com
Tel/Fax: +98-34-31325011

ABSTRACT

The purpose of this study was to examine investigating relationship betweenthe GSTM1, GSTT1 and GSTP1 polymorphisms with Chronic Obstructive Pulmonary Disease (COPD) in a smoker population. Chronic obstructive pulmonary disease (COPD) has become the fourth most common single cause of morbidity, and its prevalence is increasing worldwide. It is a syndrome composed of chronic bronchitis, small airways disease (bronchiolitis), and emphysema, in varying proportions between affected individuals. There were 213 patients with COPD and 100 healthy controlsin the Iranian population. Genotyping of GSTM1 and T1 was performed using multiplex PCR. GSTP1 Ile105Val was determined using PCR-RFLP method. Data analysis included, Pearson's r Correlations, regression analysis, ANOVA analyses, Tukey, test for comparsion and SPSS software. The results showed that there is not relationship between case and control groups and the risk of COPD was not associated with GSTM1 and GSTT1 null genotype. The frequency of genotypes wild (Ile / Ile) in the patient group was 47.4% compared with 57.6% in the control group. According the results, there is not relationship between disease and GSTP1 genotype.

Key words: GSTM1, GSTT1 , GSTP1, Chronic Obstructive Pulmonary Disease (COPD**)**

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) has become the fourth most common single cause of morbidity, and its prevalence is increasing worldwide (Celli et al. 2004). It is a syndrome composed of chronic bronchitis, small airways disease (bronchiolitis), and emphysema, in varying proportions between affected individuals. This disease is commonly defined by irreversible and progressive bronchial obstruction and is associated with persistent airway inflammation (Rabe et al. 2007). Although cigarette smoking is considered the major environmental risk factor for the development of COPD, only 20–30% of chronic smokers develop severe impairment of lung function associated with this pathogenesis (Lokke et al. 2006). These individual differences in susceptibility to tobacco smoke injury may be related to genetic factors.During the last three decades, research studies reported that the imbalance of the protease–antiprotease and the oxidant–antioxidant systems is the major factor causing emphysema and COPD (Church and Pryor 1985).Associations have been reported between the GSTM1 gene deletion and the pathogenesis of lung cancer (Nazar-Stewart et al. 1993), bladder cancer (Bell et al. 1993), and emphysema (Harrison et al. 1997). GSTT1 conjugates glutathione and various potential carcinogens, including halomethanes (Pemble et al. 1994), which are present in cigarette smoke, and its null allele has been suggested as a risk factor in several diseases (Chen et al. 1996). The data regarding the involvement of both GSTM1 and GSTT1 in COPD are still controversial. Several studies have shown that the GSTM1 gene is associated with the development of COPD either alone or in combination with other genes, such as microsomal epoxide hydrolase (mEPHX). No relationship has been reported, however, between COPD and the homozygous null genotype of GSTT1 (Harrison et al. 1997; Chen et al. 1996; Baranova et al. 1997; Yim et al. 2000; Cheng et al. 2004; Lu and He 2002; Xiao et al. 2004; Z˘ idzik et al. 2008; Hersh et al. 2005).

MATERIALS AND METHODS

There were 213 patients with COPD and 100 healthy controls, all unrelated, involved in this case–control study. In this study, patients candidate for COPD were selected in Kerman hospital in Iran. Inclusion criteria for COPD were chronic airway symptoms and signs such as coughing, breathlessness, wheezing, and chronic airway obstruction.COPD phenotype identification was based on chest radiographic and high-resolution computerized tomography density findings.

DNA Preparation

For genotyping, 5 ml blood was drawn into an EDTA tube and stored at -20C until DNA extraction was carried out. Genomic DNA was isolated from whole peripheral blood using the Salting out (Jawdat et al. 2010).

GST Genotyping

GSTM1, T1 and P1 polymorphisms were detrmined as descripbed before by authors (Mandegary, 2011). To examine the polymorphisms of GSTM1 and GSTT1 a simultaneous amplification of genes of interest in the same reaction was performed using a multiplex polymerase chain reaction (PCR) as described in the literature (Arand et al. 1996). The primer pairs for each gene were, for GSTM1, 5-GAACTCCCTGAAAAGCTAAAGC-3 and 5-GTTGGGCTCAAATATACGGTGG-3; for GSTT1, 5-TTCCTTACTGGTCCTCACATCTC-3 and 55 TCACCGGATCATGGCCAGCA-3. GSTP1 Ile105Val was determined using PCR-Restriction fragment length polymorphism (RFLP) (Mandegary et al, 2011). Briefly, a 285-bp fragment of GSTP1 gene was amplified using the following primers: sense 5' CCA ACC CCA GGG CTC TAT G 3' and antisense: 5' CTG GGA CAA GAC ACA ACC TGC 3'. The PCR product was subsequently digested by 1 unit of the restriction enzyme *Alw26I* (Takara, Japan) for 12 h at 37°C. The digests were then analyzed by electrophoresis in 3% agarose gels and visualized by ethidium bromide staining. The digestion of the PCR products yielded bands of 285 bp for Ile/Ile wild-type genotype, 195 bp and 85 90 bp for the Val/Val genotype, and three bands of 285 bp, 195 bp, and 90 bp for the Ile/Val (Heterozygots) genotype.

Statistical Analysis

Data analysis included, Pearson's r Correlations, regression analysis, ANOVA analyses, Tukey' test for comparsion and SPSS software (package ofSpss / pc + + ver18).

RESULTS AND DISCUSSION

Of the 313 subjects enrolled in the study, 213 were COPD patients (male:female ratio 163:50;smoker: Non smoker ratio 162:41) and 100 were healthy controls (male:female ratio 100:0; smokers: Non smokerratio 90:10). Both patients and controls had a similar body mass index (BMI) . There was no significant difference between patients and controls in terms of pack years of cigarette smoking.

DeterminationGSTM1 and GSTT1 Polymorphisms

Figure 1 shows thedifferent polymorphisms of GSTM1, GST T1 after the Multiplex PCR, including GSTM1, GSTT1 and primers Abl.

GSTM1 Polymorphisms in Case and Control groups

Table 1 shows the results GSTM1 Polymorphisms in case and control groups.GSTM1 null genotype patients was higher than the control group. According the results, there is not relationship between Case and Control groups and the risk of COPD was not associated with GSTM1 null genotype. These results are in good agreement with result Çalkoglu et al (2006), Chan-Yeung et al (2007).Mehrotra et al (2010)reports GSTM null genotype frequency in the patient group was 28% compared with the control group (32%) and GSTM1 null genotype was not associated with risk of COPD.RamziLakhdar (2010) survey relationship between polymorphisms of GSTM1, GSTT1 and COPD disease using Multiplex-PCR method and reports GST M1 null genotype frequencypatients was higher than the control group.

GSTT1 Polymorphisms in Case and Control groups

Table 2 shows the results GSTT1 Polymorphisms in case and control groups. GSTT1 null genotype control was higher than the patients group. According the results, there is not relationship between Case and Control groups and the risk of COPD was not associated with GSTT1 null genotype.Mehrotra et al (2010) reports the frequency of GSTT1 null genotype was significantly higher than the control group patients (40% to 14%) and showed that genotype with susceptibility to COPD may be related GSTT1 null. Lakhdar et al (2010) the survey relationship between polymorphisms of GSTM1, GST T1 and COPD disease and there is not relationship between Case and Control groups.Mehrotra et al (2010) finding the frequency of GSTT1 null genotype was significantly higher than the control group patients (40% to 14%) and GSTT1null genotype may be associated with COPD.RamziLakhdar (2010) survey relationship between polymorphisms of GSTM1, GSTT1 and COPD disease using Multiplex-PCR method and reports there is not relationship between Case and Control groups and the risk of COPD was not associated with GSTT1 null genotype.

GSTP1 Polymorphisms in Case and Control groups

Table 3 shows the results GSTP1 Polymorphisms in case and control groups. The frequency of genotypes Wild (Ile / Ile) in the patient group was 47.4% compared with 57.6% in the control group. According the results, there is not relationship between disease and GSTP1 genotype. These results are in compliant with result and Chan-Yeung et al (2007) reports there is not relationship betweendistribution of genotypes of GSTM1, GSTT1 and GSTP1 in patients

and control group. These results are in disagreement with Mehrotra et al (2010) finding Exon genetic (5GSTP1) polymorphism may be associated with COPD disease because the genotype of GSTP1 Ile105 was seen in COPD patients.

REFERENCES

1. Arand M, Muhlbauer R, Hengstler J, Ja¨ger E, Fuchs J, Winkler L, Oesch F (1996) A multiplexpolymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase
2. Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW (1993) Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 that increases susceptibility to bladder cancer. J Natl Cancer Inst 85:1159–1164.
3. Çalıkoglu M, Tamer L, Aras NA, Karakaş S, Ercan B.The association between polymorphic genotypes of glutathione S-transferases and COPD in the Turkish population.Biochemical genetics. 2006;44(7-8):307-19.
4. Celli BR, MacNee W, ATS/ERS Task Force (2004) Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. EurRespir J 23:932–946.
5. Chan-Yeung M, Ho S, Cheung A, So L, Wong P, Chan K, et al. Polymorphisms of glutathione S-transferase genes and functional activity in smokers with or without COPD. The International Journal of Tuberculosis and Lung Disease. 2007;11(5):508-14.
6. Cheng SL, Yu CJ, Chen CJ, Yang PC. Genetic polymorphism of epoxide hydrolase and glutathione S-transferase in COPD.European Respiratory Journal. 2004;23(6):818-24.
7. Church DF, Pryor WA (1985) Free radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 64:111–126.
8. Harrison DJ, Cantlay AM, Rae F, Lamb D, Smith CA (1997) Frequency of glutathione S transferase M1 deletion in smokers with emphysema and lung cancer. Hum ExpToxicol 16:356–360.
9. Lakhdar R, Denden S, Knani J, Leban N, Daimi H, Hassine M, et al. Association of GSTM1 and GSTT1 polymorphisms with chronic obstructive pulmonary disease in a Tunisian population. Biochemical genetics. 2010;48(7-8):647-57.
10. Lokke A, Lange P, Scharling H, Fabricius P, Vestbo J (2006) Developing COPD: A 25 year follow up study of the general population. Thorax 61:935–939.
11. Mandegary A, Rostami S, Alimoghaddam K, Ghavamzadeh A, Ghahremani MH. Gluthatione-S-transferase T1-null genotype predisposes adults to acute promyelocytic leukemia; a case-control study. Asian Pac J Cancer Prev. 2011;12(5):1279-82.
12. Mehrotra S, Sharma A, Kumar S, Kar P, Sardana S, Sharma J. Polymorphism of glutathione S-transferase M1 and T1 gene LOCI in COPD. International journal of immunogenetics. 2010;37(4):263-7.
13. Nazar-Stewart V, Motulsky AG, Eaton DL, White E, Hornung SK, Leng ZT, Stapleton P, Weiss NS (1993) The glutathione S transferase mu polymorphism as a marker for the susceptibility to lung carcinoma. Cancer Res 53:2313–2318.
14. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB (1994) Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271–276.
15. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB (1994) Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271–276.
16. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J (2007) Global initiative for chronic obstructive lung disease: global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease; executive summary. Am J RespirCrit Care Med 176:532–555.

17. Xiao D, Wang C, Du MJ, Pang BS, Zhang HY, Xiao B, Liu JZ, Weng XZ, Su L, Christiani DC (2004) Relationship between polymorphisms of genes encoding microsomal epoxide hydrolase and glutathione S-transferase P1 and chronic obstructive pulmonary disease. Chin Med J 117:661–667.
18. Yim JJ, Park GY, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG (2000) Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. Thorax 55:121–125.
19. Z ˇidzik J, Slaba´ E, Joppa P, Kluchova´ Z, Dorkova´ Z, Skyba P, Habalova´ V, Salagovic J, Tka´cova´ R (2008) Glutathione S-transferase and microsomal epoxide hydrolase gene polymorphisms and risk of chronic obstructive pulmonary disease in Slovak population. Croat Med J 49:182–191.
20. Jawdat N. Gaaib , Adnan F. Nassief. And Akeel H. Al-Assi.(2010).Simple salting – out method for genomic DNA extraction from wholeblood.Tikrit Journal of Pure Science 16 (2):9-11.

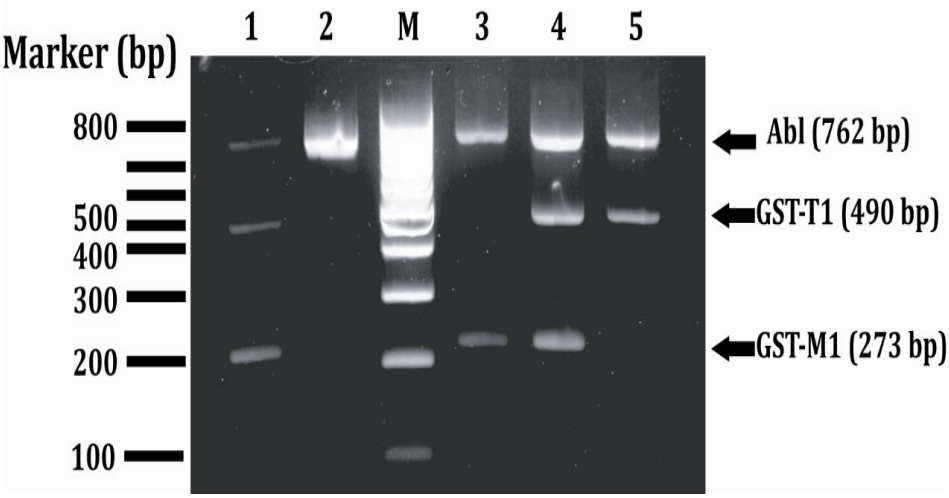

Figure 1:Multiplex-PCR to determine homozygous deletion alleles GSTM1 and GSTT1

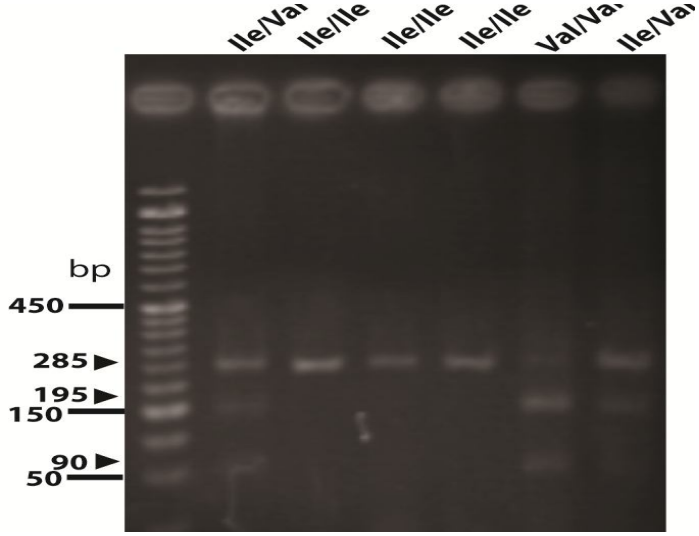

Figure 2:PCR-RFLP to determine GSTP1 genotypes

Table 1:The frequency genotypes of GSTM1 in Case and Control groups

GSTM1 genotype	Case		Control		OR (95% C.I)	P-value
	count	%	count	%		
GST M1-Null	96	45.1	42	40.8	(0.839-1.475)	0.471
GSTM1	117	54.9	61	59.2	(0.758-1.134)	

OR: Odd Ratio CI: Confidence interval

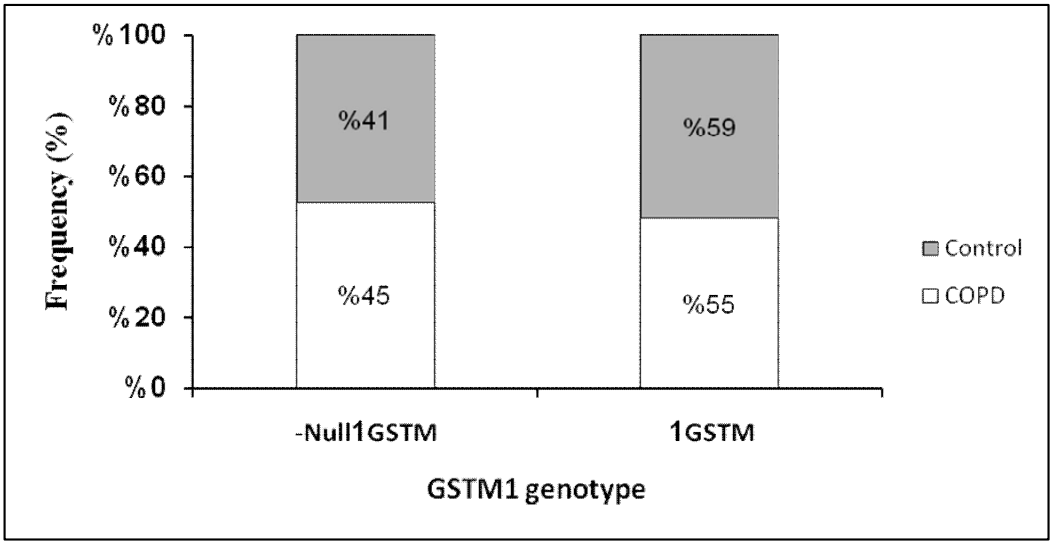

Figure 3:The frequency genotypes of GSTM1 in Case and Control groups

Table 2: The frequency genotypes of GSTT1 in Case and Control groups

GSTT1 genotype	Case		Control		OR (95% C.I)	P-value
	count	%	count	%		
GST T1-Null	68	32.1	37	35.9	(0.646-1.234)	0.49
GSTT1	144	67.9	66	64.1	(0.893-1.259)	

OR: Odd Ratio CI: Confidence interval

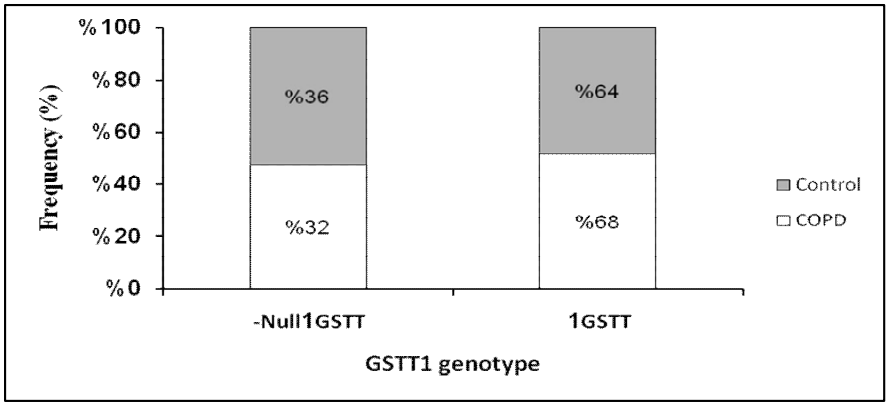

Figure 4:The frequency genotypes of GSTT1 in Case and Control groups

Table 3:The frequency genotypes of GSTP1 in Case and Control groups

GSTTP1 genotype	Case		Control		OR (95% C.I)	P-value
	count	%	count	%		
Ile/Ile	74	47.40	49	57.6	Reference	**0.143**
Ile/Val	64	41	32	37.6	(0.43-1.31)	**0.323**
Val/Val	18	11.5	4	4.7	(0.12-1.05)	**0.061**

OR: Odd Ratio CI: Confidence interval

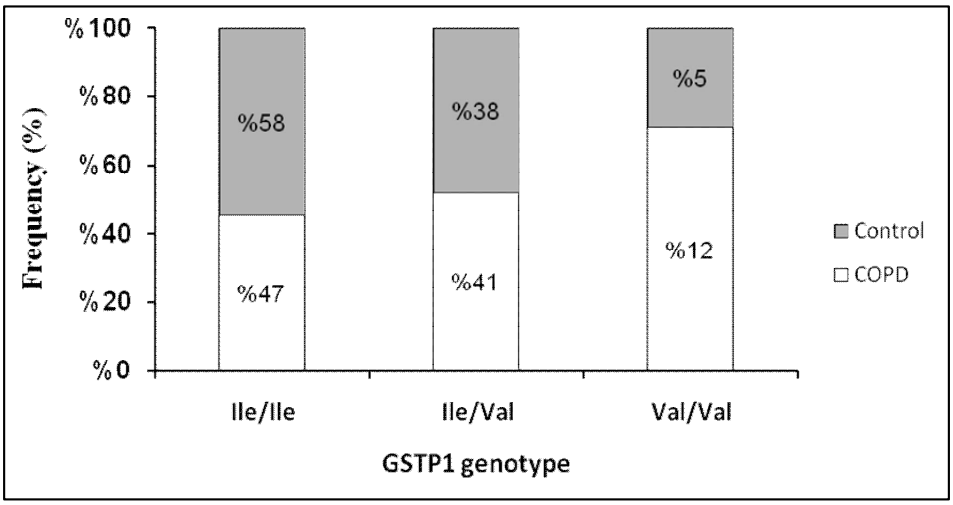

Figure 5: The frequency genotypes of GSTP1 in Case and Control groups