RESEARCH ARTICLE

Effect of Soil Type, Source of Phosphorus and Application of Sulfur with Thiobacillus on Soil Phosphorus Fractions

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ABSTRACT

In order to investigated the effects of soil type, source of P fertilizers, sulfur with thiobacillus applications on Olsen-P and inorganic P fractions in the experiment a greenhouse study as a 2*4*2 factorial experiment in a complete randomized design was performed with three factors of soil at 2 levels, P application at 4 levels and sulfur with thiobacillus at 2 levels and with 3 replications. The treatments were incubated for one year under FC moisture conditions. Effect of soil levels, P fertilizer sources and sulfur with thiobacillus application on inorganic P forms was significant during this time. Ca_2-P increased in Ammonium phosphate and triple super phosphate treatments. Increase of Ca_8-P in ammonium phosphate treatment was more than triple super phosphate treatment and sulfur with thiobacillus application decreased P fixation in soil after 80 days.

Key words: Inorganic P fractions, Available phosphorus, Thiobacillus

INTRODUCTION

Malakooti (2000) reported that of 750 thousand tons phosphate fertilizer consumed, annually, 250 thousand tons is produced in the country and the rest is imported from outside the country, due to the abundance and cheapness presence phosphate mines in the country, we can determine the way to replace this fertilizer instead of conventional phosphate fertilizers. Normality, Olsen phosphorus is not high in this matter. But the results showed of research conducted by Ghani and et al., (1994); Tandon and Prakash (1988); Tate (1995); Kilham (1994) that can be increased Olsen phosphorus in this material by applying methods. One of these methods is the use of sulfur with thiobacillus.

Besharati (1999); Besharati and Saleh Rastin (2000) reported that Bacteria of the genus thiobacillus is most important and common in type of sulfur-oxidizing bacteria in most agricultural soils. These bacteria used to produce a kind of phosphate fertilizer that is called the BioSuper. BioSuper is a mixture of soil phosphate and sulfur that is obtained by adding bacteria thiobacillus. Stevenson and Cole (1999) Reported that sulfuric acid obtained from the oxidation of sulfur reacts with the soil phosphate and it produces more soluble materials such as di and mono-calcium phosphate. Delua et al (1989); Pathiratna et al (1989) reported that Sulfuric acid obtained from rapid oxidation of sulfur leads to solubility of insoluble phosphate compounds. Lotf Allahi et al (2001) reported that the highest yield of corn obtained from treatment containing phosphate soil, phosphate soil, organic matter, sulfur and thiobacillus to amount 8.72 ton/ha. They suggested that instead of phosphate fertilizers imported, we can use from phosphate soil produced in country if organic matter and bacteria thiobacillus added to it.

Stevenson and Cole (1999) reported that sulfuric acid that is produced from Oxidation of sulfur by sulfur oxidizing bacteria and reacts with phosphate soil and produces more soluble materials such as Ca2-P and Ca-P. Kaplan and Orman (1998) in greenhouse and field trials in calcareous soils found that sulfur consumption increase the crop yield and the amount of iron, zinc, manganese that increase phosphorus absorbed by sorghum and this increase in yield is probably due to increased Olsen of iron, zinc, phosphorus, manganese and copper in comparison with the control.

Abd- Elmonem and Amberger (2000) reported that in investigate two types of rock phosphate in acidic soils, calcareous and alkaline with the use of organic matter with sulfur, increases extractable with sodium bicarbonate but the solubility in alkaline and calcareous in soils are considerably less than acidic soil, increased available phosphorus can be due to Oxidation of sulfur by bacteria and convert it to sulfuric acid, which is due to increase the solubility soil Phosphate.

Muchovej et al (1989) in research examined the effects moderators' phosphate rock with Thiobacillus bacteria, sulfur and organic matter on the changes different forms of phosphorus. After 7 weeks couch, Phosphorus content linked with aluminum Increased and phosphorus linked with organic material maximized after 3 weeks and then decreased. Sulfur and Thiobacillus treatment reduced the soil pH to 4 and became due to increase the level of phosphorus. After couch, sorghum cultivation was carried out for 30 days. Of course, in the absence of phosphorus fertilizer and use of triple Super phosphate fertilizer and finally, comparing the performance of the plant in without fertilizer application treatments to control - Triple super phosphate fertilizer application - not significant. It should be noted that the application of phosphate soil alone is not suitable for calcareous soils because naturally and due the abundance of calcium ion, phosphorus reaction with active calcium of soil goes toward apatite formation and free phosphorus in the soil cannot be released.

MATERIALS AND METHODS

This experiment were performed a factorial and in a randomized complete design with 16 treatments with three replications in controlled conditions include soil type in 2 levels, high lime (35%) and low lime (6.7%), Phosphorus fertilizer with four levels (without fertilizer, use of 100 $mg.kg^{-1}$ Pure phosphorus from triple super phosphate source, use of 100 mg kg^{-1} pure phosphorus from Ammonium phosphate source, use of 100 $mg.kg^{-1}$ Pure phosphorus from phosphate soil source), activated sulfur with 2 levels (2% by weight of soil), use of thiobacillus (10% by weight of sulfur) and (without sulfur application activated). After applying treatments, Soil moisture was kept in FC %80, inorganic forms of phosphorus studied after 80 days and results were analyzed by statistical software. Characteristics of the studied soils are presented in (Table 1).

Total P in soil was measured after the digestion with $HClO_4$-H_2SO_4 (Bao, 2000). Olsen-P was determined by extraction of air-dry soil with 0.5 mol L^{-1} $NaHCO_3$ at pH 8.5 (Olsen et al., 1954). Soil P inorganic fractionation was carried out according to a fractionation scheme for calcareous soils based on methods described by Jiang and Gu (1989). In the P

inorganic fractionation scheme, soil P inorganic was divided into six fractions as Ca_2-P, Ca_8-P, Al-P, Fe-P, Occluded P, and Ca_{10}-P.

RESULTS

(It should be noted in all the tables was cited the analysis of variance the numbers mean squares).

Effect of soil type, P fertilizer sources and applying activated sulfur by thiobacillus on mineral forms of phosphorus in the soil. Table 2 showed the results of analysis of variance the effects of soil types, sources of phosphorus fertilizer and activated sulfur by thiobacillus on mineral forms of phosphorus in the soil. These results show that soil type has significant effect on all forms of mineral phosphorus in 1% level, effect consumption of activated sulfur with thiobacillus on forms Ca_8-P in 1% level. And interactions soils and consumption of Activated sulfur with thiobacillus only on Fe-P was significant in 1%. Sources of phosphorus fertilizer have a significant impact on all forms of mineral phosphorus in the soil in 1% level. But interaction between soil and sources of phosphorus fertilizer is significant only on the Ca_2-P, Occluded P and apatite. The interaction of sulfur application activated with thiobacillus and fertilizer sources on forms of Ca_2-P, Ca_8-P was significant at 1% and for Fe-P was significant in 5%. The interaction of soil, Activated sulfur with thiobacillus and the sources of phosphorus fertilizer on form of Ca_2-P were significant in 1%. In the case of Fe-P, Occluded P and Ca_{10}-P significant in 5% level. The interaction of soil, Activated sulfur with thiobacillus and the sources of phosphorus fertilizer also on forms Ca_2-P is significant at 1% level and in the case of Fe-P, occluded P and Ca_{10}-P is significant in 5% level.

Figure 1 shows the percentage change of Ca_2-P the studied soils after 80 days and Figure 2 shows the percentage change the Ca_8-P in soil. Figure 3 show the percentage change in Al-P the studied soils and Figure 4 shows the percentage change in Fe-P the studied soils. S_0T_0, S_1T_1, with and without consumption of activated sulfur with Thiobacillus; P_1, P_2, P_3, P_4, without consumption the phosphorus fertilizer, phosphorus fertilizer application from source of ammonium phosphate, phosphorus fertilizer application from source of super phosphate triple, phosphorus fertilizer application From source of Apatit.

Figure 5 shows the percentage change in apatite in the studied soils and Figure 6 shows the percentage change in total forms of mineral phosphorus in the soils studied.

Table 3 shows comparison of the mean effects of soil type, sources of phosphorus fertilizer, thiobacillus with sulfur on forms of mineral phosphorus after 80 days.

P_1 ،P_2 ،P_3 ،P_4: without consumption the phosphorus fertilizer, phosphorus fertilizer application from source of ammonium phosphate, phosphorus fertilizer application from source of super phosphate triple, phosphorus fertilizer application From source of Apatit.

DISCUSSION

Table 2, sources of phosphorus fertilizer on all forms of mineral phosphorus in soil has a significant effect in 1% level. But the interaction of soil and sources of phosphorus fertilizer is significant only in the case of Ca_2-P **,** occluded P and apatite. The interaction of sulfur application with thiobacillus and sources of fertilizer in case forms of Ca_2-P and Ca_8-P is significant in 1% level and in the case of iron phosphate is significant in 5% level. in Figure 1, when phosphorus fertilizer application From source of super phosphate triple and ammonium phosphate greatly has increased the amount Ca_2-P in soil and if we use From sulfur with thiobacillus, An increase these figure From phosphorus becomes less. Also, if you do not use from phosphorus fertilizer, amount di-calcium decrease that quite is rational.

Figure 2 shows the percentage change the Ca_8-P in soil. As can be seen, lack use of phosphorus fertilizer and use of phosphorus fertilizer from source of super phosphate triple and phosphate soil in soil (1) with high lime led to increase Ca_8-P in soil. While in soil (2), Lack use of phosphorus fertilizer and use of phosphorus fertilizer from source of Ammonium phosphate and phosphate soil leads a reduction in Amount Ca_8-P in soil. And use of sulfur with thiobacillus significantly reduce from amount Phosphorus fixation in this form. Here have been observed that Triple super phosphate fertilizer in the form of Ca_8-P be fixed much more abundant compared to ammonium phosphate. Thus, effect of mount lime and initial phosphorus in the soils studied in fixation of phosphorus to form Ca_8-P to be determined. In figure 3 in all fertilizer treatments, amount fixation this form of phosphorus has increased and use of thiobacillus with sulfur decrease large amount from phosphorus Fixation in the form of aluminum phosphate and this effect in soil (1) with low initial storage of phosphorus and lime is appear more. As can be seen in Figure 4, in all treatments has increased the amount of iron phosphate and this increasing in fertilizer application is more than the absence of fertilizer. The use of sulfur with thiobacillus especially in the soil (1) with high lime cause an increase fixation of phosphorus in the form of iron phosphate that perhaps this be due to the increase solubility of iron oxides in the soil and its reactivity with absorbable phosphorus. As is shown in Figure 5, in time absence use of phosphate fertilizers, amount fixation of phosphorus in this form is reduced but use of thiobacillus and sulfur with different sources of fertilizer leads an increase Fixation of phosphorus the form of apatite. Probably a slight decrease in pH after 80 days will cause the dissolution of lime in the soil and calcium ions react rapidly with soil Olsen phosphorus and this reaction in soil (1) with high lime is higher than soil (2) with low lime. Figure 6 shows the percentage change in total mineral forms of phosphorus in soils studied. Since a large percentage of all forms of mineral phosphorus in soil form apatite therefore, process of change in all forms of mineral phosphorus in soil has very similar to change the apatite in soils studies but considering that phosphorus used from source of triple super phosphate, especially in soil (1) with high lime has been Fixation the form of Ca_8-P; thus, an increase the total mineral forms of phosphorus when use of fertilizer can be attributed to this formation of phosphorus. In table 3, most amount Ca_2-P in soils (1) and (2) is obtained at time use of phosphorus from source of super phosphate triple and ammonium phosphate that shows a close relationship between Olsen phosphorus with this form of phosphorus. Talibodin et al (1958) obtained process of reactions convert monocalcium phosphate in calcareous soils over time as follows:

monocalcium phosphate $\rightarrow$ dicalcium phosphate $\rightarrow$ octacalcium phosphate $\rightarrow$ Hydroxyapatite

In soil number one, use of thiobacillus with sulfur has reduced the formation of di-calcium phosphate significantly. The most amount of octacalcium phosphate is in soil (1) in terms of use of triple super phosphate and not use of thiobacillus with sulfur which shows the effect of fixation of lime in Fixation of phosphorus in this form. In severe calcareous soils, Primary products and increase monocalcium phosphate will be Ca_8-P but in other soils, Ca_2-P over time becomes Ca_8-P (Strong and Rasz, 1970). With increasing pH and lime occurs faster conversion Ca_2-P to Ca_8-P (Bell and Blake, 1970). By comparison sources of fertilizer can be seen that fixation of phosphorus from the source of fertilizer the super phosphate triple form Ca_8-P in both soils is more than a source of ammonium phosphate fertilizer and use of thiobacillus with sulfur reduces significantly from amount of fixation, particularly in soil (2).

Most amount of aluminum phosphate was found in the soil (2) and under conditions use of ammonium phosphate fertilizer but this amount is not significant difference with conditions use of triple super phosphate. Use of sulfur with thiobacillus has reduced the fixation of phosphorus to form ammonium phosphate, particularly in the soil (1). Most amount of iron phosphate obtained in conditions of use of triple super phosphate and ammonium phosphate fertilizers and use of sulfur with thiobacillus have not a dramatic impact on phosphorus fixation in this form. Amount phosphorus occluded P in soil not been significantly affect the biological phosphorus fertilizers. Magoeir et al (2000) at reviews Phosphorus Forms in modified soils by biological fertilizers from 8 soil with histories of management measured the amount of soluble phosphorus, aluminum phosphate, iron phosphate, occluded phosphorous, calcium phosphate and total phosphorus. Increasing biological fertilizers due to increase in total phosphorus, iron phosphate and aluminum phosphate. Most amount of apatite obtained in condition use of phosphorus fertilizers especially phosphate apatit. Extreme differences in the amount of apatite in soil (2) than soil (1)

is due to the initial amount of high this form from phosphorus in soil (2). Also effect of use of also effects of sulfur with thiobacillus was observed in the increase fixation of phosphorus to form apatite and after 80 day's use of phosphorus fertilizer identified significantly fixation the phosphorus to form apatite.

CONCLUSION

The impact use of Sulfur with thiobacillus became significant on mineral forms of Ca_2-P, Ca_8-P and aluminum phosphate and reduced the phosphorus fixation to the form.
Use of different sources of phosphate fertilizers had significant effect on mineral forms the phosphorus.
Use of sulfur with thiobacillus caused the sharp decline in soil pH after one year from applying treatment, So that this reduction was strongly influenced by amount of lime in soil.

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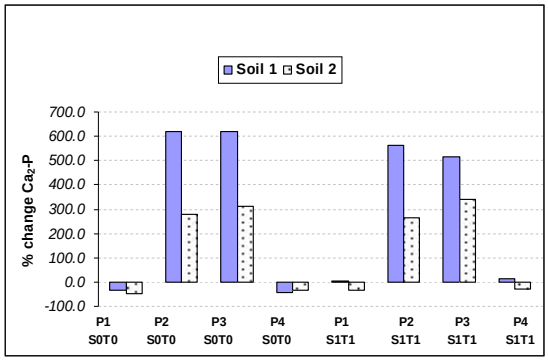

Figure 1. Percentage change in Ca_2-P the studied soils after 80 days

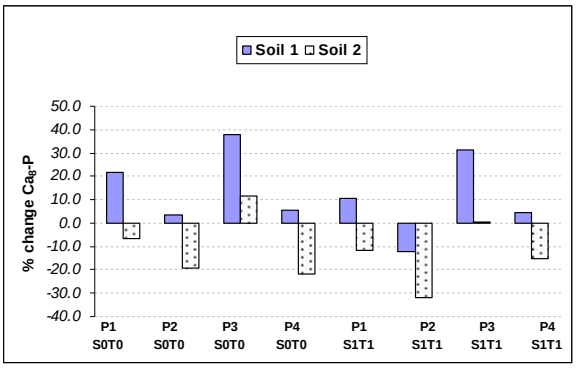

Figure 2. Percentage change in Ca_8-P the studied soils after 80 days

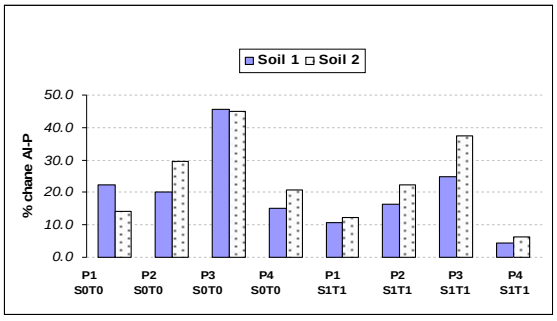

Figure 3. Percentage change in Al-P the studied soils after 80 days

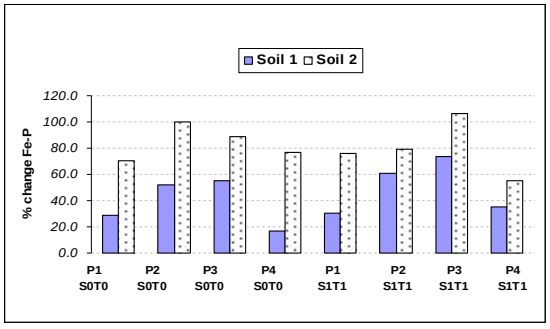

Figure 4. Percentage change in Fe-P the studied soils after 80 days

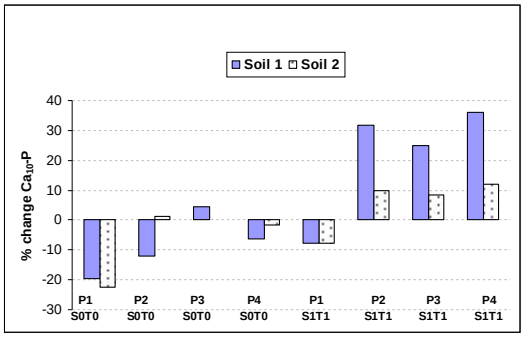

Figure 5. Percentage change in apatite the studied soils after 80 days

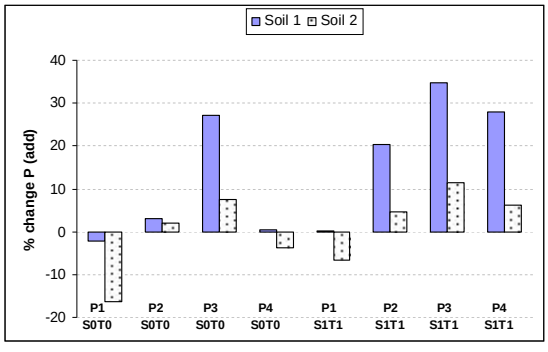

Figure 6.Percentage change in All forms of mineral phosphorus the studied soils after 80 days

Table 1. Chemical properties and forms of mineral the soils studied

Soil No.	pH	E.C dS m^{-1}	Clay	TNV activated	T. N.V	K available	Zn available	Mn available	Cu available	Fe available
			%			mg kg^{-1}				
1	8.1	1	26	19	35	260	0.44	15	2.2	15
2	7.9	0.77	21	3.2	7.6	363	0.18	16	2	16

Continue of table 1

soil No.	C.E.C cmol.kg^{-1}	SAR	Ca_2-P	Ca_8-P	Al-P	Fe-P	Occluded P	Ca_{10}-P	Total-P	Soluble-P	Olsen-P
			mgkg^{-1}								
1	22	6.2	5.2	185	28	16	15	293	790	0.47	5.1
2	31	2.1	9.2	212	34	15	14	697	1018	3.4	8.8

Ca_2-P dicalcium phosphate, Ca_8-P Octacalcium phosphate, Al-P, aluminum phosphate, Fe-P iron phosphate, O-P Occluded P, Ca_{10}-P apatite

Table 2. Results of variance analysis the effects of soil type, sources of phosphorus fertilizer, sulfur with thiobacillus on mineral forms phosphorus in the soil

sources of change	df	Ca_2-P	Ca_8-P	Al-P	Fe-P	Occluded P	Ca_{10}-P
Repeat	2	9.303^{*}	34.52^{ns}	1.53^{ns}	2.68^{ns}	50^{ns}	5439
Effect of soil type	1	20.67^{**}	5399^{**}	917^{**}	250^{**}	627^{**}	1774659^{**}
Effect of Thiobacillus	1	0.03^{ns}	2356^{**}	104^{**}	4.23^{ns}	84^{ns}	82917^{**}
Interaction of Soil and Thiobacillus	1	8.417^{ns}	65.6^{ns}	1.13^{ns}	20.4^{**}	194^{ns}	186^{ns}
Effect sources of phosphorus fertilizer	3	3795^{**}	10785^{**}	166^{**}	84.4^{**}	324^{**}	36488^{**}
The interaction of soil and sources of phosphorus fertilizer	3	17.52^{**}	11.26^{ns}	11.95^{ns}	2.75^{ns}	429^{**}	3173^{*}
The interaction of Thiobacillus and sources of phosphorus fertilizer	3	11.73^{**}	600^{**}	4.94^{ns}	7.62^{*}	108^{ns}	1634^{ns}
The interaction of Soil and Thiobacillus and sources of phosphorus fertilizer	3	15.2^{**}	105^{ns}	5.45^{ns}	8.15^{*}	155^{*}	2413^{*}
Experimental error	30	2.562	77	9.18	2.16	56.2	712.5

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

Table 3. Comparison of the mean the effects of soil type, sources of phosphorus fertilizer, thiobacillus with sulfur on the forms of mineral phosphorus in soil (Duncan 5%)

treatment	Ca_2-P	Ca_8-P	Al-P	Fe-P	Occluded P	Ca_{10}-P
	mg kg^{-1}					
$S_1T_0P_1$	3.5 ef	225 cd	34.1 efg	20.75 ij	11.3 cd	236 g
$S_1T_0P_2$	37.3 ab	192 fg	33.6 efg	24.45 fg	13.33 bcd	258 fg
$S_1T_0P_3$	37.5 ab	255 a	40.7 cd	25 efg	25 bc	307 f
$S_1T_0P_4$	3 f	195 fg	32.1 fg	18.9 j	19 bcd	275 fg
$S_1T_1P_1$	5.4 def	204 ef	30.9 fg	21 ij	12 cd	271 fg
$S_1T_1P_2$	34.5 c	162 i	32.5 fg	26 defg	11 cd	387 e
$S_1T_1P_3$	32 c	242 ab	34.8 efg	28 cd	27.3 b	367 e
$S_1T_1P_4$	5.85 def	193 fg	29.2 g	21.8 hi	45.1 a	400 e
$S_2T_0P_1$	4.85 def	198 ef	39 cde	26.05 defg	15.4 bcd	540 d
$S_2T_0P_2$	34.8 bc	171 hi	44.4 abc	30.6 ab	16.3 bcd	705 b
$S_2T_0P_3$	37.6 ab	237 bc	49.6 a	28.9 bc	5.5 d	698 b
$S_2T_0P_4$	5.95 def	166 hi	41.3 cd	27 cdef	18.7 bcd	686 bc
$S_2T_1P_1$	6.05 de	188 fg	38.4 de	27 cdef	14.7 bcd	642 c
$S_2T_1P_2$	33.6 c	145 j	41.9 bcd	27.4 cde	13.3 bcd	767 a
$S_2T_1P_3$	40.2 a	213 de	47 ab	31.6 a	7.33 d	756 a
$S_2T_1P_4$	6.55 d	180 gh	36.4 def	23.8 gh	15.1 bcd	781 a

S: soil type,T_1 and T_0 : With and Without sulfur application along thiobacillus

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RESEARCH ARTICLE

Effect Fiber of Rye on Physicochemical, Texture and Sensory Properties of Low Fat Burger

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ABSTRACT

In this research, duo to increasing importance of producing low fat meat products such ashamburger and in order to developing new and safe product with low fat and cholesterol contentand high fiber, effect of different levels of Secalecereale(Fiber of Rye)as a fat replacer onphysicochemical, textural and sensory properties of low fat hamburgers were compared with highfat control samples. Control with 14/41% fat and 0Fiber of Rye, and low fat hamburgers with %2and%4, %6, 8a% and %10Rye fiberwere produced. Results showed that it is possible toreduce the fatcontent of the hamburger samples up to 35/25 percent. There wasa direct correlation between Fiber of ryelevel and moisture,ash and cooking loss contentafter cooking. Sensory analyses were highly favorable and all samples gained acceptable score.Findings of all textural parameters revealed that low fat samples with 4%Fiber of Ryewere more similar to control than others and could imitate textural properties of high fat products.Statistical analysis of the data was carried out by using computer programme Spss19 statistical software package. To analyze the carrying out of sensory tests the Kruskal-Wallis test was used.Given the significant differences between treatments the Duncan test at 0/05 level was used to compare averages. In this study, a new formulation for producing a healthy meat product withlower fat and higher dietaryFiber of Ryeas a functional food was introduced.

Key words: Fiber of Rye, Physico-Chemical properties, Sensory properties and low-fat hamburger

INTRODUCTION

The prevalence of diseases related to high fat diets have caused the increase of consumers' demand as well as recommendations of international scientific organizations on consuming low-fat food products such as low fat hamburger. The main objective of this research is producing a low fat meat product as a healthy and functional food.

The meat we eat is an integral component in our diet. In the food pyramid, they are considered as protein food long with other food category like poultry, fish and eggs.Undoubtedly, meat is a major source of food proteins with high biological value in many countries. Meat is also an excellent source of some essential fats, soluble vitamins and minerals, and all these components have specific function to our body. But recently, negative campaign about muscle foods, and their possible health hazard effects, shows that consumers are increasingly interested about health oriented functional meat products. According to them, food they consume should not only taste better but also be attractive, safe and healthy, sincetime constraints prevent them from spending enough time for exercise to keep them fit. Consumers are getting educated with nutritional information and are beginning to understand. This powerful influence of diet on health and well being, increasing scientific evidence confirms that specific components in diet may tend off certain chronic diseases such as cardiovascular diseases, variouscancers and neurological disorders (Ames et al., 1993). It has revitalized the interests not only in consumer, but also among researchers and meat food productprocessors to develop formulated products, which are "natural, functional and nutritional" as well. Functional meat products either possess nutritional ingredients that improve health or contain lesser quantity of harmful compounds like cholesterol, fat etc (Yue, 2001; Diplock et al., 1999). These products are generally produced by reformulation of meat by incorporatinghealthproducingingredients like variety of fibers, protein, polyunsaturated fatty acids (PUFA), antioxidants etc. Meat products that contain dietary fibers are excellent meat substitutes due to their inherent functional andnutritional effects (Hur et al., 2009; Kumar et al., 2010).

Further, dietary fiber intake through meat substituted with fruits, vegetables and certain grains is associated with reductions in plasma and LDL-cholesterol, reduce the risk of major dietary problems such as obesity, coronarydiseases, diabetes,gastrointestinal disorders, including constipation, inflammatory bowel diseases etc(Schneeman, 1999). Besides health benefit effects, dietary fiber supplementations increase the bulk andprevent cooking loss in meat products with no or fewerchanges in textural parameters by enhancing waterbinding capabilities and carries great economicaladvantages for both the consumers and processors(Grigelmo-Miguel et al., 1999).

Fiber is suitable for meat products preparation because of its water retention property, decreases cooking lossand neutral flavor. Dietary fibers isolated from various plants have diverse functional properties namely solubility, viscosity, gel forming ability, water-binding capacity, oil adsorption capacity, and mineral and organic molecule binding capacity, which affect product quality and characteristics (Tungland and Meyer, 2002).Dehydrated fruit, vegetable and cereal fiber can be used in the food industry as functional ingredient with excellent results (Viuda-Martos et al., 2010).

MATERIALS AND METHODS

Preparation of Fiber of Rye

Rye bran with high fiber content , quality control laboratory were approved by a reputable manufacturer of flour mills , then rye bran groundIn order to reduce the microbial load and inactivation of enzymes, bran produced Was agitated at 121 C for 20 minutes(Galdeano& Grossman ,2005)

Milled rye bran was treated with hydrogen peroxide under alkaline conditions. The resulting suspension was centrifuged at 10000 * g round Sediment obtained after the final washby the filter was smoothSolid compounds were obtained from centrifuge was smooth .And they were dried by oven for 24 hours at 40 C . (Could, 1989:Inglett, 1998.)

Preparation of Burger Samples

The burger formulae were formed using a patty marker (stainless steel model "Form") to obtain round discs 10 cm diameter and 0.5 cm thickness. After preparation of each formula, the burger samples (each sample was 50 g) were packed in polyethylene bags and were stored immediately in a deep freezer at -18°C for analyses before and after cooking. Basal burger formula included minced beef, salt, 0. onion, flour, mixed spices and iced water(Ali, M. A. ,2008)The suspensions of Rye fiber (2,4,6,8,10%), were added to basal formula to prepare different low-fat burger treatments.

Chemical properties

Moisture, fat, ash of the product were determined bynational standards.of the Number 745,742,744.

Instrumental colour

Thecolour of the samples was measured using the Hunter LAB system with a tristimulascolorimeter _Hunterlab, Model No.D-25, Fairfax, USA.. The colorimeter was used with a5 mm diameter viewing area and Hunter L, aL and bLvalues were measured. Three readings were taken from each burger.

Mechanical textural analysis

Texture analysis system was used forthe objective evaluation of textural parameters (Desmond, et al, 1998).

Cooking losstest

Frozen hamburger samples for 20 minutes was defrostedthen weighed and after fryingthe samples were weighed again and using the formula, cooking loss can be calculated

(100* Initial weight / weight final- initial weight) = percent cooking loss

Sensory evaluation

Sensory evaluation (appearance, color, texture, taste, odor, mouth feelandoverallacceptability) of the8-point Hedonic methods and questionnaire were valuated by 8 trained panelists. (Desmond, et al, 1998).

Statistical analysis

Statistical analysis of the data was carried out by using computer programme SPSS19 statistical software package.

RESULTS AND DISCUSSION

The results of the present research show that total fat in the hamburger is 14/41 % , that in of the low-fat burgers, this amount reduces to 5/08 percent , which represents a 35/25 % reduction of total fat in the final product. Since producing low fat products is main purpose of this study, the finding of this study conform with the results of Ingett and warner In 1997, in which they reduced the amount of fat in the meat grinder from 11 to 7 percent using corn z-trim. Applying dietary fiber in the Frankfurter (25% fat) Sausage, lmo-Miguel Girge et al. in 1999 produced low-fat sausages of 5 & 25 percentGarcia et al. (2002) reported 60% decrease of fat in dried sausage fermentation formulated with dietary fiber compared to the controlling sample.

The results show that due to high water absorption of fiber rye, the amount of humidity in samples containing replaced fat increases significantly compared to controlling samples(P<0/05) and with the increase of the amount of fiber rye, the amount of humidity also increases. It is in a way that the samples containing 10% rye fibers, has the highest amount of humidity. This increase of humidity is because by replacing oil with water, some of total fat in the final product decreases, which comply with the findings of Girgelmo -Miguelet al. (1999). In this study by increasing the amount of water, fat content decreases.

The amount of the ash samples containing 10% fiber rye are more than other samples. There is a direct relation between Ryefiber contentsandash content. The increase of fiber content results a meaningful increase of ash (P <0/05). The cause of such increasecan beattributedtothe amount ofminerals infiberrye. In a study, Yilmaz (2005)increases the ash content of beef fat cells samples in which wheat bran is replaced for fat; and the more fiber content, the more ash content. Furthermore, the replacement offatwithfiber rye indicatesstatistically that it has a significant effect on weight decrease after baking, and low-fat samples have more weight reduction is insignificant and doesn't have negative effect on the product.

Also, all histological features affected by the amount of fat and fiber rye content in the samples results in the increase of hardness in 10%-fiber rye sample; and 5/08 fat percentage is significantly higher than the other samples. .This isdue to the lossoffandincreaseof water in the product. Also the features ofchewinessandelasticity in higher-fatcontrolsample are significantly higher than low-fat samples, because fat causes the softness of tissue and reduction of the required force to chew the food. However, changing the amount of fiber rye in low-fat samples doesn't cause any significant change in the two histologically feature that mentioned above, and all the samples treated by fiber rye are similar in chewiness and elasticity (P>0/05).

Increase of fiber rye content and reduction of fat decreases cohesion, samples containing 10% fiber rye show the least cohesion. Replacing fat with inulin, Dad.mondevzaet al (2000) studies on the effect ofinulinin histological featuresof sausages samples, the result of the mentioned study are similar to the findings of the present study.

According to the color test results, the amount of b in low-fat treatment with fiber rye, There was no significant difference compared to controlling sample. In the study of Claus and Hunt, although the adding dietary fiber caused some changes in b content, however, it is not significant which conform to the results of this study. The L content of low-fat treatment does not have a significant difference with that of controlling sample.

Therefore, addition of rye fiber doesn't cause the darkness or lightness of hamburger samples. In fact, Lisafactorthatis a subject to thebrightness of thesamples' color. Function ofwater contentandfat amount of hamburgerformulationcanaffect onthe amount ofL.Research showsthatfatreductionmakes the productdarker; whileincrease of water percentage makes it brighter,thatin the treatments ofthisstudy,bothof them occurred, which may cause no change in L. adding 3.5% of a type of fiber compound to the controlling sample containing 10% fat,

Huntand Claus (19910 observe no significant difference in L content comparing to the controlling sample compared to controlling samples.

Finally, according tothe resultsofsensorytests, themean of sensory features of hamburger treatment areacceptable.Treatments from general acceptance aspect, treatments shows a significant difference compared to the controlling sample (p <0/05).

CONCLUSION

In this study, Based onresults of Physicochemical test, usingthe formulationofhamburgerFiber of ryecanreducethe amount offat inthe samplesRata35.25percent and a new formulation for producing a healthy meat product withlower fat and higher dietary fiber as a functional food was introduced.

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Effect of Fiberof Ryeon physicochemical, texture and sensory properties of burger

Samples containing 10% fiber rye	Samples containing 8% fiber rye	Samples containing 6% fiber rye	Samples containing 4% fiber rye	Samples containing 2% fiber rye	control	physicochemical and sensory properties of burger
65/4±0/519^{f}	62/3±0/0115^{e}	59/32±0/0100^{d}	57/42±0/0152^{c}	54/22±0/0152^{b}	51/12±0/0200^{a}	Moisture
2/5±0/100^{f}	2/2±0/100^{e}	1/8±0/577^{d}	1/7±0/577^{c}	1/3±0/577^{b}	1/1±0/100^{a}	Ash
5/08±0/577^{f}	7/2±0/739^{e}	8/38±0/804^{d}	10/47±1/075^{c}	12/41±1/101^{b}	14/41±.0/868^{a}	Fat
0/0818 ^{f} 36/21±	34/43±17/871 ^{e}	32/47±17/186^{d}	30/13±0/0251 ^{c}	/32±0/0100^{b} 28	26/71±0/0100^{a}	Cooking loss
27/25±0/0100a	27/35±0/0152a	27/45±0/0057a	27/52±0/0208a	27/93±0/0115 a	27/95±0/0057 a	L* factor in color samples
9/92±0/0057b	9/89±0/0100b	9/87±0/0100b	9/79±0/0100b	9/74±0/000b	9/67±0/0057b	b* factor in color samples
5/18±0/057c	5/35±0/000c	5/41±0/0057c	5/65±0/0100c	5/72±0/0152c	5/85±0/0057c	*a factor in color samples
4/126±0/001^{d}	3/344±0/002^{c}	2/985±0/001^{b}	1/935±0/001^{a}	2 ± 0/001 ^{a}	1/871±0/0005^{a}	TextureHardness
0/126±0/0005^{d}	0/135±0/0043^{d}	0/147±0/0005^{d}	0/244±0/0005^{c}	0/316±0/0005^{b}	0/420±0/0036^{a}	TextureSpringiness
0/471±0/0006^{d}	0/527±0/0001^{d}	0/634±0/0006^{d}	0/712±0/0006^{c}	0/876±0/0001^{b}	0/912±0/0006^{a}	TextureGumminess
0/136±0/000^{d}	0/143±0/000^{d}	0/146±0/00100^{d}	0/226±0/00100^{c}	0/370±0/0037^{b}	0/451±0/0005^{a}	TextureChewiness
5/00±0/000 a	4/66±0/577 a	3/66±0/577 b	3/66±0/577 b	2/66±0/577 c	2/33±0/577 c	Flavor
5/00±0/000 a	4/66±0/577 ab	4/00±0/000 ab	3/66±0/577 bc	3/00±0/000 bc	2/66±0/577 c	Shape
5/00±0/000 a	4/66±0/577 a	3/66±0/577 b	3/66±0/577 b	2/66±0/577 c	2/33±0/577 c	Texture
5/00±0/000 a	4/66±0/577 ab	4/00±0/000 b	3/00±0/000 c	2/66±0/577 c	2/33±0/577 c	Color
5/00±0/000 a	4/00±0/000 b	3/66±0/577 b	3/66±0/577 b	2/66±0/577 c	2/66±0/577 c	Total acceptation

Different letters within the same column indicate significant differences ($P < 0.05$)

RESEARCH ARTICLE

Comparison Inspection of Pseudomonas Aeruginosa Infection in Different Organ (Heart, Ovarium, Liver and Feces) in Poultry Slaughter House in Urmia with Bacterial Culture Method

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ABSTRACT

Pseudomonas aeruginosa is a common bacterium that can cause disease in animals, including humans. It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonization's occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal. Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is implicated in hot-tub rash. The aim of this study was to evaluate and compare the bacterium Pseudomonas aeruginosa infection in various organs (heart, liver, ovary, bowel) using a bacterial culture in poultry slaughter house Urmia. After sterilization equipment, sampling took from Urmia abattoir which consists of 30 steps, or a month. At each stage 4 (liver, heart, ovary and feces) were collected after 24 h of incubation and enrichment medium agar Macconkey, BHA and differential medium was used. From 1440 samples, 85 stool, 30 heart, 60 liver and 40 cases of ovarian Pseudomonas isolated.

Key words: Pseudomonas, infection, bacterial, contamination, poultry

INTRODUCTION

It is a Gram-negative, aerobic, coccobacillus bacterium with unipolar motility. An opportunistic human pathogen, P. aeruginosa is also an opportunistic pathogen of plants.[6] P. aeruginosa is the type species of the genus Pseudomonas (Migula). Pseudomonas aeruginosa is a common bacterium that can cause disease in animals, including humans. It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal.[1] Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is implicated in hot-tub rash. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills. On 29 April 2013, scientists in Rensselaer Polytechnic Institute, funded by NASA, reported that, during spaceflight inside the International Space Station, P. aeruginosa bacteria seem to adapt to the microgravity and the biofilms formed during spaceflight exhibited a column-and-canopy structure that has "not been observed on Earth".

An opportunistic, nosocomial pathogen of immunocompromised individuals, P. aeruginosa typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections. The aim of this study is Comparison inspection of pseudomonas aeruginosa infection in different organ (heart, ovarium, liver and feces) in Poultry slaughter house in Urmia with bacterial culture method.

MATERIALS AND METHODS

The medium enrichment, selective and differential medium was acting in the following way:
A) Primary culture and enrichment:
Selenite F broth
B) Selective medium:
BHA
SS agar
MacConkey agar
C) Differential media and tests:
Simmons Citrate, Urea Agar, SIM, TSI, MR_VP
Catalase and Oxidas tests

The sampling procedure was as follows:

After autoclave and sterilization equipment, sampling over a month (30 nights) in a way that at each stage of the four parts (heart, liver, ovary, bowel), poultry slaughter house Urmia sampling was done. Samples were inoculated in Selenite F broth medium and after 24 h of incubation transferred to MacConkey agar, SS agar and BHA. After 24h incubation at 38-43 ° C suspected colonies were observed.

Cultivation in Differential media:

After 24 h incubation in MacConkey agar, SS agar and BHA suspected colonies were transferred to differential media and Catalase and Oxidas tests on suspected colonies was done.

RESULTS

From 1440 samples, 85 feces, 30 heart, 60 liver and 40 cases of ovarian Pseudomonas isolated.

DISCUSSION

In this study pseudomonas aeruginosa infection were studied. In this study most of pollution in feces with 85 pcs and lowest pollution in heart with 30 pcs. Bacterial infection of meat inspected in worldwide. For example Fatemeh Ashrafi in 2009 reported that 40% of meat and poultry meat infected with Yersinia (Fatemeh Ashrafi, 2009).

In another research Laura FRANZETTI and Mauro SCARPELLINI in 2007 with title Characterization of Pseudomonas spp. isolated from foods showed that 6 cluster of Pseudomonas isolated from foods (Laura FRANZETTI and Mauro SCARPELLINI, 2007).

Foodborne diseases are a serious and growing problem. Foodborne diseases cause an estimated 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year. Experts estimate that the yearly cost of all foodborne diseases in this country is $5 to $6 billion in direct medical expenses and lost productivity. Salmonella infections alone account for $1 billion yearly in direct and indirect medical costs. [1] However, many cases of foodborne illness go unreported.

Since 1906, meat inspection has been limited to organoleptic methods, which rely on detecting changes in the sight, smell, or feel of the tissues. But this does not detect foodborne microorganisms. For the past several years, the National Academy of Sciences has recommended that a more complete inspection be instituted, which would include microbiological monitoring, since many contaminants could be present in numbers high enough to cause disease without altering the sight, smell, or feel of the carcass, or even without causing visible disease in living cows, pigs, or chickens.

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Table1: positive and negative samples from 1440 samples

part	Total sample	positive	negative
ovarium	360	40	320
feces	360	85	275
heart	360	30	330
liver	360	60	300
total	1440	215	1225

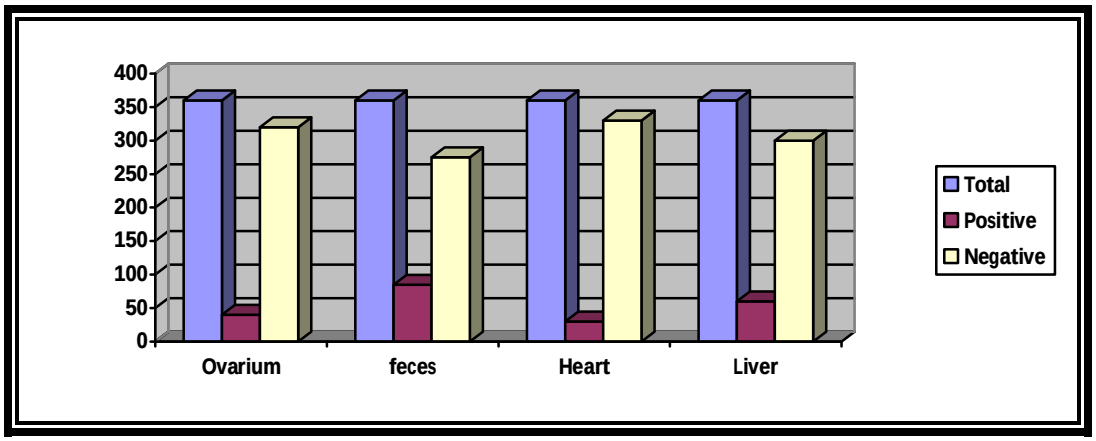

Figure 1: positive and negative samples from 1440 samples

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RESEARCH ARTICLE

Detection of Shiga Toxin-Producing Escerichia Coli by PCR from Raw Cow Milk in Jahrom, South of IRAN

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ABSTRACT

Introduction and Purpose: Strains of Shiga toxin-producing Escherichia coli (STEC) are the main factors of diseases transmitted by food and one of the most important agents of intestinal infections. Since food materials produced by livestock, especially cow milk, are one of the main sources of Escherichia coli and EHEC strains, this research intended to detect of shiga toxin-producing Escerichia coli from raw cow milk.Methods: Three hundred and twenty two raw milk samples were randomly taken from dairy farms and stores in Jahrom, south of IRAN in late March 2014. After identifying the bacteria and determining the serotypes, the serologically positive Escherichia coli isolates were separated and the shiga toxin-producing Escerichia coli were identified using specific primers of the stx1 and stx2 genes and PCR, and the pattern of antibiotic resistance was determined using the CLSI protocol.Results: Only 26 (18.84%) of the 312 milk samples were infected with pathogenic serotypes, in that 4 of them were O157:H7 serotype and the others were Non-O157:H7.Twenty-two (80%) of the 26 serologically positive E. coli isolates had the stx1 gene and one of them(3.80%) had both stx1 and the stx2 genes. The most and the least sensitive antibiotics were ceftazidime and doxycycline, respectively. Conclusions: Detection and identification of shiga toxin-producing Escerichia coli in 7% of the milk samples in this region shows that health officials must perform more preventive operations on dairy products and training people for prevention of infections.

Key words: Escerichia coli, milk, shiga toxin, stx1 and stx2 genes

INTRODUCTION

Escherichia coliis agram-negative, non-spore forming (4) and motile bacteria of the family Enterobacteriaceae (1,2,3) used as an indicator for determining of fecal contamination of water and food materials. E. coli is a typical member of the normal bacterial flora of the digestive systems in human and other warm-blooded animals that usually do not affect human health but can, in some cases, cause acute digestive diseases (2, 4).Based on pathogenic factors, these bacteria have been classified into several pathotypes named EHEC(Entero Haemorrhagic Escherichia Coli), EPEC(Entero Pathogenic Escherichia Coli), EAEC(Entero Aggregative Escherichia Coli), ETEC(Entero Toxigenic Escherichia Coli), and STEC(Shiga Toxin producingEscherichia Coli) or VTEC(VerotoxigenicEscherichia Coli)(19) . STEC is one of the most pathogenic bacteria in this family (2) and the first human pathogen identified in 1982 (6, 12).Since then, more than 200 STEC serotypes have been isolated from animals, foods, and other sources. E.coli O157H7 is the most important STEC serotype (7) and causes seroius syndromes such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (10, 11, 5, 7, and 9). Food products such as raw milk and unpasteurized cheese are the known infection source of E. coliO157:H7 (13).Laboratory studies using animal models have shown several virulence factors named stx1, stx2, eaeA, and hlyA are involved in the pathogenicity of STEC strains (7). The most important of these factors are the Shiga toxins (5) that are usually encoded on plasmids and transposons and carried by bacteriophages (2) and, due to their conspicuous effects on vero cells, are also referred to as verotoxins (6, 7, and 8). The polypeptide Shiga toxins consist of the two toxins, stx1 and stx2, encoded by stx1 and stx2 genes in the pathogenicity islands of the bacterial genomes (4). In E. coli O157H7, which is the most common STEC serotype, stx2 is more frequently than stx1 (8), and it has also been reported that, under laboratory conditions, stx2 has more cytotoxic effects on endothelial cells than stx1 and, hence, is more involved in causing HUS (8).Although it seems endothelial cells are the main target for stx, there are evidences that these toxins have biological effects on other cell types including renal tubular cells, monocytes, platelets, and mesenchymal cells (8). Recently, mortality worldwide resulting from prevalence of the STEC serotype has emphasized the role of this microorganism in public health. Therefore, more demand has been made to study the prevalence of these organisms and to develop new and accurate methods for detecting them in food materials such as meat and dairy products (15,16, 21, and23). Therefore, considering the probable presence of E. coli and shiga toxin - producing strains in food materials especially milk and dairy products, and because of the great importance of these strains in human sanitation and health, this study was designed for detecting of shiga toxin-producing Escherichia coli from raw cow milk in Jahrom, south of IRAN.

METHODS

Isolating ofE. coli from raw milk

In late March 2014,312 raw milk samples were collected randomly from dairy farms and supermarkets in Jahrom. The samples (200 ml) were transferred in sterile containers at 4 C° to the laboratory. The samples were first cultured in nutrient broth for initial enrichment. Nutrient and selective culture media such as blood agar, EBM agar, and MacConkey agar were then used for microbial tests in order to detection ofE. coli. After staining and employing biochemical tests such as oxidase, MR,VP, TAI, Citrate, SIM, Sorbitol-MacConkey, and urea, E.coli were identified, and commercial anti-serum was used by the latex agglutination method according to the protocol of the manufacturing company (Baharafshan) to determine E. coli serotypes (which were kept at -70 C° for future tests).

Identification of strains containing the stx1 and stx2 genes

After extracting of DNA using kits manufactured by the Cinnagen Company, PCR with the specific primers (Table 1) was performed to identify E. coli O157H7 strain and the strains producing the stx1 and stx2 genes,. The PCR reaction took place in an Eppendorf thermocycler under the temperature conditions of initial denaturation for five minutes at

95 C°, followed by 35 cycles including denaturation for one minute at 94 C°, annealing for one minute at 94 C°, extension for one minute at 72 C°, and finally, final extension for five minutes at 72 C°. PCR products were electrophorosed for further investigation.

Sterile distilled water as negative control and E. coliO157:H7 as the positive control were used in the PCR reactions. Primers were synthesized by the Takapouzist Company, and lyophilized master mix vials manufactured by this company were used for performing PCR reactions. TheSPSS software was used to analyze results obtained in the study.

RESULTS

One hundred and thirty eight (44.23%) of the 312 samples were infected with E. coli and, after serotype determination, it was found only 26 (18.84%) of the isolates were pathogenic serotypes. In that four (15.38%) isolateswere O157:H7 serotype and 22 (84.62%) were Non-O157:H7 serotype. The results of PCR showed that 21 (80%) of the 26 serologically positive E. coli isolates had the stx1 gene, and 1 isolate (3.80%) both the stx1 and stx2 genes. The PCR products of the stx1 and stx2 genes on agarose gel exhibited the 180 bp and 255 bp bands, respectively (Figures 1 and 2).

Results obtained from the PCR of the stx1 and stx2 genes indicated that 3 (75%) of the 4 isolates with E. coliO157:H7 serotype had the stx1 gene and 1 isolate both the stx1 and stx2 genes, and 17 (85%) of the isolates from those of the E. coli Non-O157:H7 serotype had the stx1 gene but no isolates had the stx2 gene.

Moreover, results of antibiotic sensitivity test showed that all E. coliO157:H7 serotypes were sensitive to the antibiotics ceftazidime, ofloxacin, trimethoprim-sulfamethoxazole, and ampicillin, resistant to doxycycline and cefotaxime, and however, were relatively high sensitivity (75%) to gentamicin. Among the E. coli Non-O157:H7 serotypes also, the highest sensitivity was to ceftazidime, ofloxacin, and gentamicin, and the lowest to doxycycline and cefotaxime (Table 2). The pattern of antibiotic resistance indicated that, the maximum sensitivity was to ceftazidime (92.9%), and the minimum to doxycycline (7.1%).

DISCUSSION

In recent years, theshiga toxin-producing strains of E. coli, especially the serotype O157:H7, are the main agents of intestinal bacterial infections those have caused epidemics in some parts of the world. Therefore, researchers and other people involved in health affairs have shown interestingon these strains, and considerable researches has been conducted on infectionswith food materials including milk and dairy products.In a study carried out by Shariatifar et al. in Mashhad, from 120 (24%) of milk samples were infected with E. coli, 76 (15.2%) of which were identified as sorbitol negative (O157:H7) strains (22). Although the percentage of infected milk in this research was lower than ours, but the percentage of samples containing pathogenic strains was higher. This difference may be due to the methodology, because the identification of pathogenic strains using the serotype method is more accurate compared to sorbitol negative cultures. Therefore, our results seem to be more reliable.

Kargar et al. (23) carried out a study in three different areas in Jahrom and found that 9% of the 45 milk samples were sorbitol negative. After performing confirmatory serotype tests, 3.40% of the samples were identified as E. coli O157:H7, in that all of which were resistant to penicillin, ampicillin, and novobiocin. The percentage of infected milk in this study was lower than ours, but the E. coliO157:H7 strains had a higher percentage and the pattern of antibiotic resistant was different from that we found in our research. These differences could be related to the method of sample collection and the areas selected for taking them.

In the study that Bonyadian et al. conducted on 200 milk samples in Chahar-Mahal and Bakhtiari Province, 83 (41.50%) of the samples were infected with E. coli, but none of them were E. coliO157:H7 and only 22 (11%) of Non-O157:H7 strains of E. coli were detected (20). Momtaz et al. conducted research in Shahrekord on 400 milk samples taken from cows with mastitis and found 42 (10.50%) of the samples were E. coli positive, out of which only 10 (23.8%) contained stx1 or stx2, or both, genes (27). However, in their study, random sampling was used and samples were not taken only from cows with mastitis, and of the 312 raw milk samples, 137 (44.23%) were E. coli positive (20 samples of which contained stx1 and one both the stx1 and the stx2 genes).In a study carried out by Rahimi et al. on 201 samples of milk products collected from Esfahan, Khuzestan, and Chahar-mahal and Bakhtiari, 18(8.96%) were infected with E. coli, 2% of which contained stx1 and stx2 genes (29). Farzan et al. conducted research in Shahrekord on 300 raw milk samples and dairy products collected from various areas in Iran and showed that 78 (26%) of the samples were infected with E. coli, 93.5% of which were positive for both stx1 and stx2genes (28).In Germany, M. Zschock et al. found 53.6% of the 959 collected raw milk samples were infected with the STEC strain, and study of the stx genes in the detected isolates revealed that 15 isolates (11.5%) had stx1, 60 (45.8%) the stx2 gene, and 56 (42.7%) both the stx1 and stx2 genes (17).

The results obtained by other researchers also indicated the contamination of milk and dairy products with shiga toxin- producing E. coliwas variable and could have different reasons. For example, differences in the percentages of infection could be attributed to climatic and geographical conditions, variety of the hosts, and different conditions such as those related to raising the livestock, milking method, collection and transportation method, the season in which samples were taken, the processing and production techniques used for dairy products, methods of detecting and identifying bacteria, use of molecular methods in determining types of stx genes, and the researchers skills. Nevertheless, the comparison of the results of our research with similar ones showed that these food materials have varying degrees of contamination withE. coli. ThenE. coliis one of the most important bacterial groups that contaminate milk and dairy products; and that if these food materials are contaminated with STEC strains, they can be very pathogenic and cause serious diseases. Therefore, infection with these bacteria can be prevented by keeping food materials safe; and health officials must operate greater control over these food materials and provide people with the necessary information.Moreover, results of our research suggest that there has been a change in the pattern of resistance in the bacterial strains found in Jahrom. This indicates the necessity of repeated determination of the pattern of antibiotic resistance in the bacterial strains of the region. Also, it can be a useful guideline for infectious diseasesspecialists in treating digestive infections resulting from these bacteria in the region.

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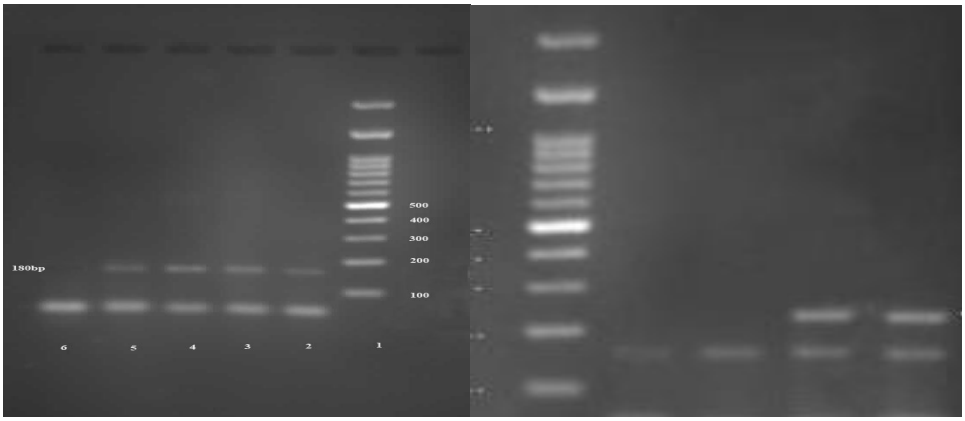

Figure 2: PCR products of the stx2 gene on agarose gel exhibited the 255bp band

Figure 1: PCR products of *stx1*(180bp) and*stx2*(255bp) genes

Table 2: The pattern of antibiotic resistance for *E. coli* isolated from raw milk

Name of the antibiotic	Resistant	Semi-sensitive	Sensitive
Trimethoprim-sulfamethoxazole	7 (25%)	2(7.14%)	24(85.71%)
Ceftazidime	1(3.57%)	1(3.57%)	26(92.9%)
Gentamicin	1(3.57%)	4(14.28%)	23(82.14%)
Ampicillin	6(21.42%)	1(3.57%)	21(75%)
Cefotaxime	8(28.57%)	11(39.28%)	9(32%)
Ofloxacin	2(7.14%)	1(3.57%)	25(89.28%)
Doxycycline	26(92.85%)	0(0.0%)	2(7.1%)
Amoxicillin	6(21.42%)	1(3.57%)	21(75%)

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RESEARCH ARTICLE

Response of Chickpea (Cicer Arietinum L.) to Different Plant Densities under Application of Lentagran Herbicide

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ABSTRACT

A field experiment was lay out in order to evaluate the effect of plant density and application of Lentagran herbicide on yield and yield components of chickpea at faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch, Boroujerd (field location: Khorramabad), Iran during the growing seasons 2013-2014. The experiment was a split-plot design based of RCBD with three replications. Treatments were three plant row spacing (20, 30 and 40 cm) in main plots and three post-emergence application of Lentagran herbicide (15, 30 and 45 day after emergence of chickpea) with control in sub plots. The results showed that the effect of plant density was significant on number of branches per plant, number of pod per plant, 100grain weight, grain and biomass yield. Effect of herbicide on number of pod per plant, 100grain weight, grain and biomass yield and HI was significant. Interaction effect of them on number of pod per plant, grain and biomass yield was significant only. The comparison of the mean values showed that 40cm row spacing had the highest number of branches per plant. However, application of Lentagran at 30 day after emergence of chickpea in 40cm row spacing treatment had the highest number of pod per plant. Also, 30cm plant row spacing had the highest 100-grain weight and application of Lentagran at 30 day after emergence of chickpea in had the highest 100-grain weight and harvest index. Moreover, application of Lentagran at 30 day after emergence of chickpea in 30cm row spacing treatment had the highest grain and biomass yield and non application of Lentagran in 20 and 40cm row spacing treatment had the lowest of them. In final our results showed that optimum plant density and application of Lentagran

herbicide in optimal dos are two of the good practice in cultivation of chickpea for achieved to higher yield in chickpea.

Key words: Chickpea, herbicide and plant density

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop of rainfed areas in semiarid/arid climate. Chickpea is a cool-season legume crop, mainly grown for human consumption with annual area sown fluctuating between 9.3 and 12 million ha over the previous decade (FAOSTAT, 2007).Average chickpea yield in different countries in the world looked such as China (4135 kg ha-1), Canada (1427 kg ha-1), USA (1391 kg ha-1) and Pakistan (785 kg ha-1). Environmental stress is a primary cause of crop loss worldwide, resulting in average yield losses of more than 50% for major crops every year (Chaves and oliveira, 2004). Chickpea yield is depend on good plant population, nutrient elements and weed management. Chickpea play a main role in the increasing of soil fertility due to its nitrogenfixing ability (Maiti, 2001).

Plant density is one of the most significant agronomic practices contributing towards grain yield, as well as other important attributes of this crop (Charles and Charles, 2006) also, is an important agronomic attribute since it is believed to have effects on light interception during which photosynthesis takes place which is the energy manufacturing medium using green parts of the plant. One of the most important cultural practices to reduce impact of weed on crops is increasing crop competitiveness by increasing plant density. The results of previous studies showed that plant density and planting pattern differently affected yield and morphological traits (Fishbach and Mulliner, 1974;). Prine (1964) reported that competing for light is the most limiting effect of plant density on plant yield. Mazaheri et al (2002) reported that enhancing plant density increased forage yield significantly. Ahmadvand et al. (2009) reported that the yield and its components of chickpea were increased significantly when chick pea density increased.

Herbicide Lentagran WP now registered for controls or suppresses redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.), nightshade spp. (*Solanum* spp.) and small flowered galinsoga (*Galinsoga parviflora* Cav.). Lentagran WP is postemergence contact herbicide and has no residual soil activity. Its mode of action involves hydrolysis to 3-phenyl-4-hydroxy-6-chloropyridazine, which then inhibits photosystem II electron transport (Zohner, 1987). Lentagran WP™ contains 45% w/w pyridate as a wettable powder formulation. The re-introduction of Lentagran WPTM will provide vegetable growers with a useful post-emergence contact-acting herbicide for the control of a range of broad-leaved weeds. Pyridate is Annex I listed and belongs to the mode of action group C3 (affects photosystem II). Half fill the spray tank with clean water and begin agitation. To minimise operator exposure, place the unopened water-soluble bag directly into the spray tank. After approximately 5-6 minutes the bag will begin to dissolve and the powder will be released. To prevent product sedimentation and to assist the dissolving process, constant agitation is required. Susceptible weeds to Lentagran include Annual nettle, Black bindweed, Black nightshade, Charlock, Fumitory, Field bindweed, Fat hen, Field penny cress, Pale persicaria and Shepherd's purse. Weeds are susceptible at the cotyledon to 4-leaf stage at the 2kg rate. Pyridate injury, which occurs as distinctive, blotchy chlorosis of treated leaves, has frequently been reported in cabbage (Miller, Hopen, 1991; Orfanedes, Masiunas, 1990; Wallace, Bellinder, 1992). The important weeds present in chickpea crop in rainfed areas are *Lathyrus aphaca* L., *Cyperus rotundus* L., *Convolvulus arvensis* L., *Medicago ploymorpha* L., *Anagallis arvensis* L., *Fumaria indica, Cynodon dactylon* (L.) Pers, and *Carthamus oxycantha* L. (Saxena, 1979). The competition of weeds with crop is mainly for available nutrients, moisture, space and sunlight etc. The quality of chickpea seed can also be deteriorated by weed infestation which creates storage problem and also affects market rate (Saxena, 1979). Weeds are of crucial importance since effective and proper weed control time will result in higher seed yields of chickpea

(khaliliaqdam et al, 2014) therefore control of weeds is very important for increasing of yield and yield components of chickpea.

Therefore this study was planned to examine response of chickpea (*Cicer arietinum* L.) to different plant density under application of Lentagran herbicide.

MATERIALS AND METHODS

A field experiment was conducted 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 response of chickpea (*Cicer arietinum* L.) Garit cultivar to different plant density under application of Lentagran herbicide. The experiment was a split-plot design based of RCBD with three replications. Treatments were three plant row spacing (20, 30 and 40 cm) in main plots and three post-emergence application of Lentagran herbicide (15, 30 and 45 day after emergence of chickpea) with control in sub plots.

To determine yield, we removed and cleaned all the seeds produced within a per square meter area in the field. The seeds were air-dried and weighed, and seed yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter and quintals per hectare. The number of pod per plant, the number of grain per pod and the number of grain per plant were determined. Replicated samples of clean seed (broken grain and foreign material removed) were sampled randomly and 100-grain were counted and weighed. The biomass production was measured on 8 plants treatment at 40 day after podding (DAP).

The harvest index was accounted with follow:

HI = (Economical yield / Biological yield)

The statistical analyses to determine the individual and interactive effects of treatments were conducted using MSTAT-C. Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

The effect of plant density on number of branches per plant was significant at 5% level (table 1), but the other treatments were not significant on it. The comparison of the mean values of the number of branches per plant (figure 1) shows that 40cm row spacing had the highest (3.6) number of branches per plant and the 20cm row spacing treatment had the lowest number of branches per plant (3.4) and the differences were significant.

The effect of plant density, herbicide and interaction between them on number of pod per plant was significant (table 1). The comparison of the mean values of the number of pod per plant for interaction between treatment (figure 2) showed that application of Lentagran at 30 day after emergence of chickpea in 40cm row spacing treatment had the highest (45) number of pod per plant and non application of Lentagran at 30 day after emergence of chickpea in 20cm row spacing treatment had the lowest number of pod per plant (10) and the differences were significant.

Table 1 shows that the effect of plant density and herbicide treatments on 100-grain weight were significant at 1% level but the other treatment was not significant on it. The comparison of the mean values of the 100-grain weight for plant density treatment (figure 4) showed that 30cm plant row spacing had the highest (30.3 g) 100-grain weight and 20cm row spacing treatment had the lowest (28.3g) 100-grain weight. Results for herbicide showed that application of Lentagran at 30 day after emergence of chickpea in had the highest (30.2g) 100-grain weight and non application of Lentagran had the lowest 100-grain weight (27.5g) and the differences were significant.

The analysis of variance showed that the effects of plant density, herbicide and interaction between them on grain yield were significant at 1% level (table1). The comparison of the mean values of the grain yield for interaction between treatments (figure 5) showed that application of Lentagran at 30 day after emergence of chickpea in 30cm row spacing treatment had the highest (200g.m^2) grain yield and non application of Lentagran in 20 and 40cm row spacing treatment had the lowest grain yield (80g.m^2) and the differences were significant.

Also results showed that the effects of plant density, herbicide and interaction between them on biomass yield were significant (table1). The comparison of the mean values of the biomass yield for interaction between treatments (figure 6) showed that application of Lentagran at 30 day after emergence of chickpea in 30cm row spacing treatment had the highest (520g.m^2) biomass yield and non application of Lentagran in 20 and 40cm row spacing treatment had the lowest biomass yield (290g.m^2) and the differences were significant (figure 6).

The analysis of variance showed that the effect of herbicide treatment on harvest index was significant (table1) and the other treatment were not significant on it. Results for herbicide showed that application of Lentagran at 30 day after emergence of chickpea in had the highest (40%) harvest index weight and non application of Lentagran had the lowest harvest index (26%) and the differences were significant (figure 7).

Mahalakshmi and Bidinger (1985) reported that different stresses at grain filling stage reduced grain yield up to 50%. In the present study results showed that optimum plant density and application of herbicides for weeds control increase yield and yield components of chickpea. Effective weed management in chickpea must also have good crop tolerance. Lentagran injury, which occurs as distinctive, blotchy chlorosis of treated leaves, has frequently been reported (Bullen et al., 1993; Miller, Hopen, 1991). Injuring is usually transitory with nonchlorotic leaves emerging subsequent to application. There have been no reported instances of yield reductions in chickpea where pyridate was applied. According to Henderson and Cairns (2002), pyridate kills weeds in broccoli, Chinese cabbage, cabbage, or cauliflower with minimal crop damage. Stall and Hensel (1994) reports about pyridate spraying in onion. In the present study application of Lentagran herbicide 30 day after emergence was more efficient on grain yield of chickpea (figure 6).

The results of this research showed that plant density and weed management is very important for optimum growth and reproduction of chickpea laid to higher grain yield and it components. For number of branches per plant, 40cm row spacing had the highest and the 20cm row spacing treatment had the lowest number of branches per plant. However, the comparison of the mean values of the number of pod per plant showed that application of herbicide at 30 day after emergence of chickpea in 40cm row spacing treatment had the highest number of pod per plant. Chickpea is a very sensitive crop to weed competition, which generally results in heavy yield loss and reduction in grain yield may vary from 23% to 87% depending on the weed species and their densities in various countries (Bhan and Kukula 1986). Weeds mainly compete with crop for nutrients, soil moisture, and sunlight by covering over crop and space (Tepe et al, 2011). Severity of yield loss depends upon weed infestation as well as climatic conditions which affect weed and crop growth (Tepe et al, 2011). Weeds can remove plant nutrients from soil more efficiently than crops (Tepe et al, 2011). Also, results reviled that 30cm plant row spacing had the highest and 20cm row spacing treatment had the lowest 100-grain weight (figure3). Results for herbicide showed that application of Lentagran at 30 day after emergence of chickpea in had the highest and non application of Lentagran had the lowest 100-grain weight (figure4).

Results of present study showed that application of Lentagran at 30 day after emergence of chickpea in 30cm row spacing treatment had the highest grain and biomass yield and non application of Lentagran in 20 and 40cm row spacing treatment had the lowest grain and biomass yield. The clean weeding increased the seed yield of chickpea by and the first 4 to 6 weeks were the most critical period for weed competition (Ahlawat et al, 1981). Bhan and Kukula (1986) founded that

the beneficial effect of reduced weed competition is apparent from the dry matter accumulation of chickpea under weed-free and weed-infested environments, which are ultimately reflected on seed yield. However, competition became more severe after 60 days (Pete et al, 2011). Hence, the first 30 to 60 days after emergence were the most critical for weed control as also indicated earlier (Saxena et al, 1976). However, Mohammadi et al (2005) founded that, chickpea must be kept weed-free between the 5-leaf and full flowering stages (24 and 48 DAE) and from the 4-leaf to beginning of flowering stages (17 and 49 DAE) at the 2 sites, respectively, in order to prevent more than 10% seed yield loss.

Application of Lentagran 30 day after mergence increase grain yield of chickpea due to decrease of competition between chickpea and other weeds and increase of leaf area and growth of it. In this concern Sharara, Faida, et al., (2006) concluded that Panther 55%Sc (0.6 L /Fed) and Arelon (1.25L/Fed) were the best effective amongst other treatments in increasing plant height and flag leaf area, of wheat plants which was estimated by up to 22%, which applying Sinal and Lentagran or Grasp and Iloxan alone showed less response in this issue Also, the increases of the wheat growth parameters by using the panther 55%Sc herbicide may be due to the severe decrease in fresh and dry weights of weeds and consequently the competition was limited and more light, water and nutrients were available to promote wheat growth. Results of this study are in agreement with those obtained by EL-Metwally (2002) and Sharara, Faida et al., (2006). Data presented in fiure (7) reveal that the application of Lentagran 30 day after mergence significantly increased harvest index. These results may be due to the dominant weeds in the chickpea field which was the broad leaved weeds than the grassy weeds as recommended by El-Metwally (2002). These increases in the growth parameters and harvest index, when Lentagran compared with non application of herbicide may be due to good control of wheat weeds and minimizing weed competition which gave good chance of wheat growth in good condition. The obtained results of the growth parameter of chickpea plants are coincided with these obtained by Sharara Faida et al., (2006).

In the present study results indicates that there were significant differences in the response of yield and yield components of chickpea to the plant densities (table 1). Different plant spacing with different plant densities generally influenced chickpea yield and yield components. 100 grain weight and grain yield increases due to better transfer of photosynthetic substances in 30cm plant row spacing. Our results showed that optimum plant density and application of Lentagran herbicide in optimal dos are two of the good practice in cultivation of chickpea for achieved to higher yield in chickpea.

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Table 1. Analysis of variance (mean squares) for yield and yield components of chickpea under different plant density and application of Lentagran herbicide

Source of variation	df	num of branches per pod	num of pod per plant	100grain weight	grain yield	Biomass yield	Harvest Index
R	3	0.032	66.7	3.9	186	1492	25.8
Herbicide(h)	3	0.106	399**	16.2**	19805**	63492*	368.7**
Error (Ea)	9	0.043	17.8	0.8	177	4981	51
Plant density(D)	2	0.184*	357**	15**	2810**	18372**	13.6
H*D	6	0.018	55*	2.3	2184**	13507*	26
Error (Eb)	24	0.026	15.3	2.4	175	2921	26.9
CV		4.45	17.9	5.3	9.2	13.3	14.7

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

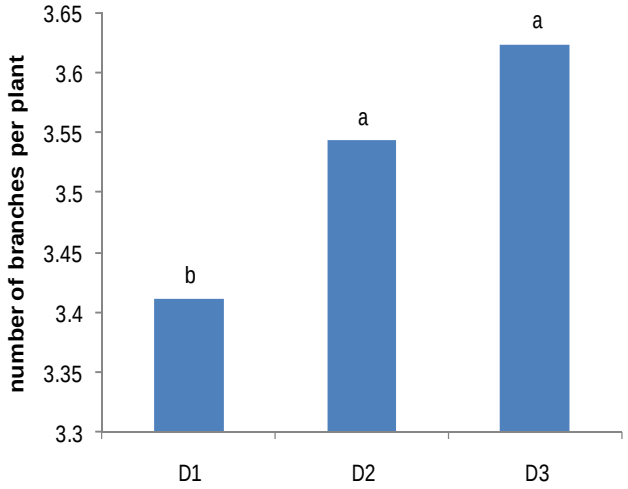

Figure 1. Effect plant density on number of branches per plant in chickpea. Means by the uncommon letter in each column are significantly different (p<0.05) (D_1= 20cm, D_2=30cm and D_3= 40cm plant row space)

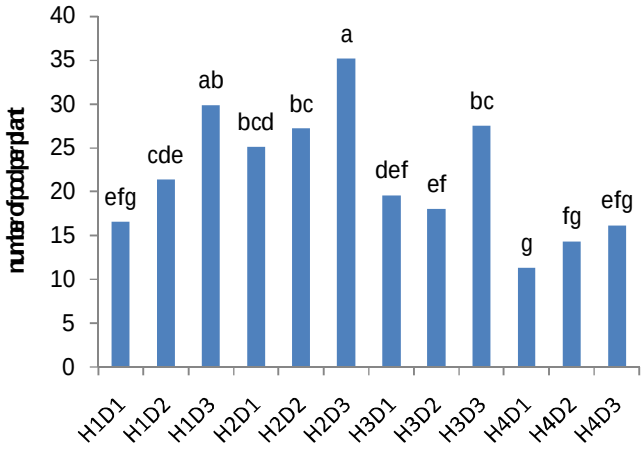

Figure 2. Interaction effect of plant density and application of Lentagran herbicide on number of pod per plant in chickpea
Means by the uncommon letter in each column are significantly different (p<0.05)
(D_1= 20cm, D_2=30cm and D_3= 40cm plant row space, H_1=12d, H_2=30d, H_3=45 day after emergence application of herbicide and H_4=non application of herbicide)

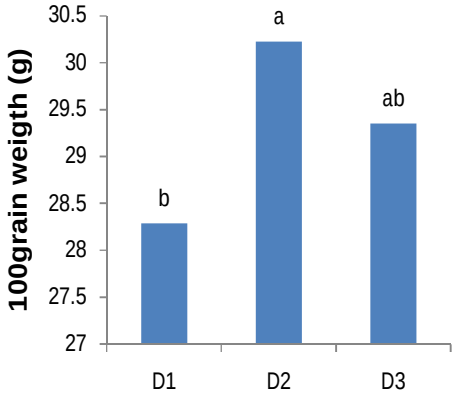

Figure 3. Effect of plant density on 100 grain weight in chickpea
Means by the uncommon letter in each column are significantly different (p<0.05)
(D_1= 20cm, D_2=30cm and D_3= 40cm plant row space, H_1=12d)

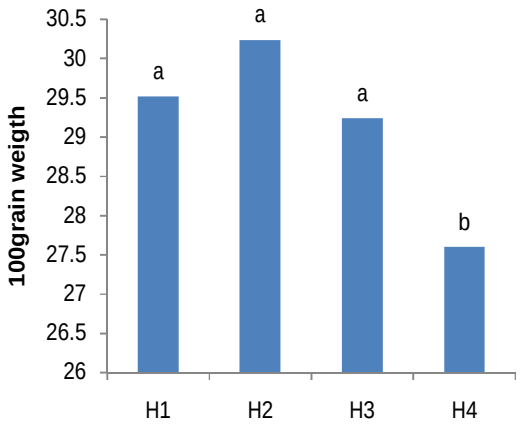

Figure 4. Effect of Lentagran herbicide on 100 grain weight in chickpea
Means by the uncommon letter in each column are significantly different (p<0.05)
(H_1=12d, H_2=30d, H_3=45 day after emergence application of herbicide and H_4=non application of herbicide)

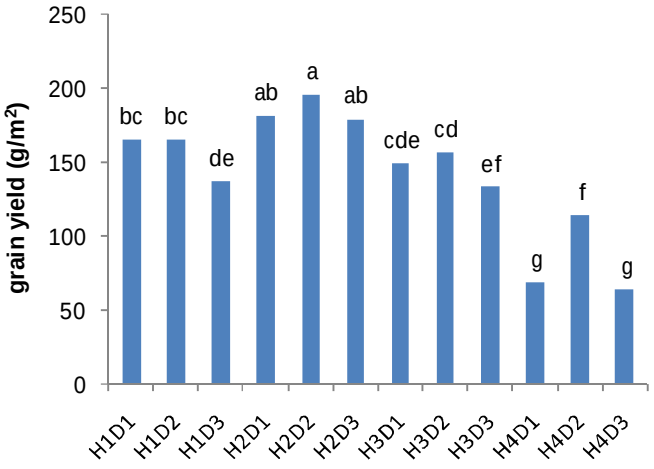

Figure 5. Interaction effect of plant density and application of Lentagran herbicide on grain yield in chickpea
Means by the uncommon letter in each column are significantly different (p<0.05)
(D_1= 20cm, D_2=30cm and D_3= 40cm plant row space, H_1=12d, H_2=30d, H_3=45 day after emergence application of herbicide and H_4=non application of herbicide)

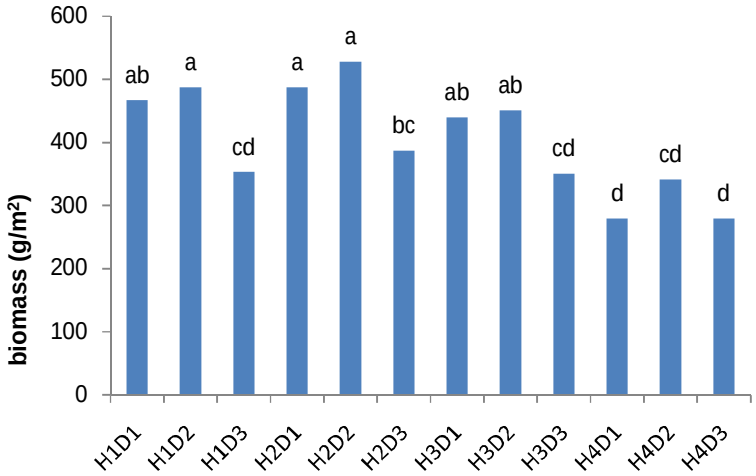

Figure 6. Interaction effect of plant density and application of Lentagran herbicide on biomass yield in chickpea
Means by the uncommon letter in each column are significantly different (p<0.05)
(D_1= 20cm, D_2=30cm and D_3= 40cm plant row space, H_1=12d, H_2=30d, H_3=45 day after emergence application of herbicide and H_4=non application of herbicide)

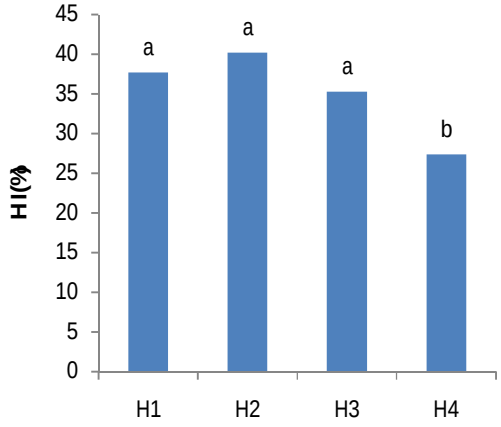

Figure 7. Effect of Lentagran herbicide on HI in chickpea.
Means by the uncommon letter in each column are significantly different (p<0.05)
(H_1=12d, H_2=30d, H_3=45 day after emergence application of herbicide and H_4=non application of herbicide)

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RESEARCH ARTICLE

Naloxone-Precipitated Morphine Withdrawal in Male Miceis Attenuated by Acute Administration of Hydroalcoholic Extract of Cymbopogon Citratus (Lemon Grass)

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ABSTRACT

The present study was conducted to investigate the effect of hydroalcoholic extract ofCymbopogoncitratus (Lemon grass) on morphine withdrawal signs. Male NMRI mice (20-30g) were rendered dependent by intraperitoneal (i.p.) injections of morphine three times daily at doses of 50, 50 and 75 mg/kg, respectively, for 3 days. On day 4, after the last administration of morphine,different doses Of Cymbopogoncitratushydroalcoholic extract(140,280 and 560 mg/kg, i.p.) and fluoxetine (20mg/kg, i.p.) administered 30 min before administration of naloxone (5 mg/kg,i.p.).Mice were observed for 30 minutes for the withdrawal signs i.e., the characteristic jumping, grooming, teeth chattering, climbing, rearing, wet dog shakes, writing and diarrhea. Results of this study indicated that all dosesofCymbopogoncitratushydroalcoholic extract ($p<0.01$, $p<0.001$ and $p<0.01$, respectively)and fluoxetine($p<0.01$) reduced the number of jumps. Moreover, all doses of extract reduced the behaviors of grooming ($p<0.05$), climbing ($p<0.05$), writhing ($p<0.05$) and diarrhea ($p<0.01$). Rearing and wet dog shakes reduced only by high dose of extract ($p<0.05$).Fluoxetine significantly alleviate the other checked signs such as grooming ($p<0.01$), climbing ($p<0.05$), writing ($p<0.05$) and diarrhea ($p<0.01$).The present study findings showed thatCymbopogoncitratusextract could attenuate morphine withdrawal signs and further studies need for clarify their exact mechanism of action.

Key words: Cymbopogoncitratus; Morphine dependence; Naloxone withdrawal signs; mice

INTRODUCTION

Opioid dependence is a chronic, relapsing brain disease that causes major medical, social and economic problems to both the individual and to society. Opioid-dependent individuals are subject to substantial health risks including overdose, transmission of infectious diseases, poor physical and mental health and frequent hospitalization (Hulse et al., 1999). Morphine is one of the most effective opioid analgesics for moderate to severe pain. However, chronic morphine use usually has unwanted adverse effects, such as analgesic tolerance and physical dependence (Bailey and Connor, 2005).Tolerance causes morphine to gradually lose its effect, and usually it requires escalating doses to produce pain relief; morphine dependence is generally characterized by serious affective and physical disorders, such as irritability, anxiety, nausea, chills, sweating, diarrhea, sneezing, and muscular and abdominal pain, upon discontinuation. These symptoms are extremely aversive and become an obstacle to abstinence treatment.Therefore, treatments that circumvent such dependence symptoms would be greatly beneficial to the abstainers (O'Connor, 2005).Furthermore, there is no approved treatment for opiate tolerance and dependence. To find a proper treatment, herbal therapy, complementary, and traditional medicine are extremelyappreciated. Hence, many researchers have been done on systems or drugs, which alleviate morphine withdrawal syndrome. These include drugs that influence dopaminergic, serotonergic(Espejo et al.,2001), adrenergic (Harris and Aston-Jones ,1993),excitatory amino acid (Rasmussen et al.,1991), nitric oxide (Dambisya and Lee,1996), GABAergic systems(Hack et al.,2003), and also herbal medicines (Abbasi-Maleki et al., 2013,2014).

Cymbopogoncitratus (DC.) Stapf (Poaceae family), commonly known as lemon grass, is a tall aromatic coarse grass of 1.5 m high. It is a monocotyledonous hypogeal perennial plant with slender sharp edged green leaves that has a pointed apex. The stem is reddish brown in color and it is attached to the bulb by stalk. The entire plant is attached to the soil by fibrous root (Burkill, 1996). Lemon grass stalks are commonly used in the cuisines of Africa, the Middle East and Southeast Asia. It is native to Sri Lanka and South India and is now widely cultivated in the tropical areas of America and Asia (Hatch,1995).Cymbopogon citratus is commonly used in folk medicine for treatment of nervous and gastrointestinal disturbances, and as antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and sedative (Santin et al., 2009). Studies on extracts from Cymbopogon citratus leaves have demonstrated antioxidant, anti-microbial and anti-fungal activities (Oloyede,2009; Pereira et al., 2009; Matasyoh et al., 2011).Therefore, The purpose of the present study was to evaluate the possible effects of hydroalcoholic extract of Cymbopogon citratuson morphine withdrawal signs in male mice.

MATERIAL AND METHODS

Preparation of extract

Fresh leaves of Cymbopogon citratus (lemon grass) was washed and air dried in the laboratory for two weeks. It was ground with excella mixer grinder and sieved with a mesh of size 0.5mm. The powdered sample obtained was stored in clean air tight containers at ambient temperature until when needed for use (Uzama, 2009 and Hassan et al.,2005). The leave powdered (50g) was placed in a 500ml conical flask. To this was added 250ml of ethanol and stirred vigorously with a magnetic stirrer. This was allowed to stand for 36h after which it was stirred and filtered. The extract was concentrated using a rotary evaporator to 50ml.The extract was cooled and stored in a refrigerator before use (Uzama, 2009).

Animals

Male NMRI mice, weighing 25-35g housed in ventilated rooms at temperature of 24 ± 2°C with a 12 h light/dark cycle and 60 ± 5% humidity. They were provided with foodand water ad libitum. All experiments were carried out in

accordance with local guidelines for the care of laboratory animals of Tabriz University ofMedical Sciences (Tabriz, Iran).

Morphine dependence

For induction of morphine dependence, the mice were treated intraperitoneally(i.p.) with morphine three times a day (10 a.m., 1 p.m. and 4 p.m.) for three days, and the doses of morphine were 50, 50 and 75 mg/kg respectively . The higher daily dose, injected at 4 p.m., was aimed at minimizing any overnight withdrawal. On day 4 they received a last dose of morphine (50 mg/kg, 10 a.m.). Groups of mice, each containing 8 animals, were animals of 8 mice were chosen randomly for the experiments.

Naloxone-precipitated withdrawal syndrome

Withdrawal signs were elicited by i.p. injection of naloxone hydrochloride (5 mg/kg) 2 h after the last injection of morphine. Counted and checked signs were evaluated during a 30 min period starting just after naloxone injection. Jumpings were counted and checked signs including diarrhea, grooming, climbing ,rearing ,teeth chattering, writing and wet dog shakes were evaluated over 30 min with one point given for the presence of each sign during each period (range of scores: 0-3) (Abbasi-Maleki et al.,2012,2013,2014).

Experimental groups

Forty mice were randomly divided into 5 groups of 8:

One chronically intraperitoneally (i.p.) morphine (as a negative control group):In this group normal saline (10ml/kg) was administered i.p. 30 min after the last dose of morphine and 30 min later, naloxone was injected. One chronically intraperitoneally (i.p.) morphine (as a positive control group):In this group fluoxetine (20mg/kg) was administered i.p. 30 min after the last dose of morphine and 30 min later, naloxone was injected. Three chronically intraperitoneally (i.p.) morphine (as a treatment group):In this group different doses of Cymbopogon citratus (140,280 and 560 mg /kg,i.p.) hydroalcoholic extract was administered i.p. 30 min after the last dose of morphine and 30 min later, naloxone was injected.

Statistical analysis

The data were expressed as mean ± S.E.M. One-way ANOVA followed by Duncan test was used for comparison of data and P values less than 0.05 were considered significant. The Mann-Whitney U test was used for comparison of checked signs data. All statistical calculations were done with SPSS for windows (SPSS 19) software.

RESULTS AND DISCUSSION

Repeated administration of morphine produced physical dependence, such that naloxone administration to the mice after the repeated morphine generated a specific set of behavioral responses including jumping, climbing, teeth chattering, writhing, wet dog shakes, grooming and rearing. In the control group that was chronically treated with morphine, significant behavioral responses (as detailed above) were induced after naloxone administration. Our resultsdemonstrated that intraperitoneal administration of different doses (140,280 and 560 mg/kg)of Cymbopogon citratus compared to control group (106.09±47.68) significantly reduced the number of jumps in morphine dependent mice (18±19.97, 12.75±10.17 and 16.5±15.67;$p<0.01$, $p<0.001$ and $p<0.01$,recpectively).In line with our results, it showed that fluoxetine(10mg/kg), as a reference drug also significantly reduced (27.8±13.95, $p<0.01$),number of jumps

(Fig. 1)(Singh et al.,2003). Several investigations report that jumping is an important sign for evaluating opiate dependence, though an effective drug should equally work against other signs of withdrawal. Furthermore, diarrhea and rearing behavior are the other common signs found during morphine withdrawal (Sepu´ lveda et al., 1999; Caille´ et al., 1999). We found in the present study that naloxone precipitated the withdrawal symptoms such as diarrhea and rearing behavior in chronic treated morphine groups. However, our results exhibit that diarrhea decreased with all doses of extract ($p<0.01$). These findings supported that previous studies and demonstrate the anti-diarrheal activity of extract. Rearing behavior decreased only by high doses of extract ($p<0.05$) (Table1). In line with our findings, Blanco et al reported that the essential oil of Cymbopogon citratus couldreduce rearing in open field test. Writing behavior was decreased with all doses of extract ($p<0.05$).Our results are in agreement with previous reports indication theanti-nociceptive activity of Cymbopogon citratus extract (Viana et al., 2000). Climbing decreased with all doses of extract ($p<0.05$).In accordance with others (Blanco et al., 2009),grooming behavior was decreased with all doses of extract ($p<0.05$)(Table1).Wet dog shakes and teeth chattering decreasedonly with high doses of extract ($p<0.05$and $p<0.01$, respectively).Fluoxetine also compared to control group significantly reduced other signs such as grooming ($p <0.01$), climbing ($p < 0.05$), writhing ($p<0.05$) and diarrhea ($p < 0.01$) (Table 1).

The exact mechanism of action of the Cymbopogon citratus extract could not be predicted from present study experiments. However, Cymbopogon citratus contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol. The essential oil consists of, mainly, citral a volatile oil with strong lemon fragrance. Citral is a mixture of two stereoisomeric monoterpenes aldehydes; the trans isomer geranial (40-62%) dominates over the cis isomer neral (25-38%) and is used in manufacture of perfumes, colored soaps and synthesis of Vitamin A(Shah et al.,2011;).In other hand, it seems that the main chemical constituent of extract, citral, was responsible for their anti-diarrheal activity (Tangpu and Yadav,2006).Costa and their colleagues report that acute treatment with lemongrass essential oil (EO) did not result in neurotransmitters (e.g. dopamine and serotonin) and it metabolites changes evaluated in cortex, striatum, pons and hypothalamus. They showed that anxiolytic-like effect of its EO is mediated by the GABAA receptor–benzodiazepine complex (Costa et al 2011).Although;these data reinforce the idea that the major compounds are not always responsible for biological activity.Furthermore, according to Silva et al. (2010) the EO from Cymbopogon citratus administered i.p. had anticonvulsant effect, by intraperitoneal route, which was blocked by the pretreatment with flumazenil (as a competitive antagonist of benzodiazepine binding, suggesting and enhancing a possible effect on the GABAergic neurotransmission system and it seems GABAergic system has key role in some biological effects of Cymbopogon citratus.

CONCLUSSION

In this study we first time showed that Cymbopogon citratus could attenuate some major signs of morphine withdrawal signs and further studies need for clarify their exact mechanism of action.

Conflict of intersts

The authors declare that they have no conflicts of interest.

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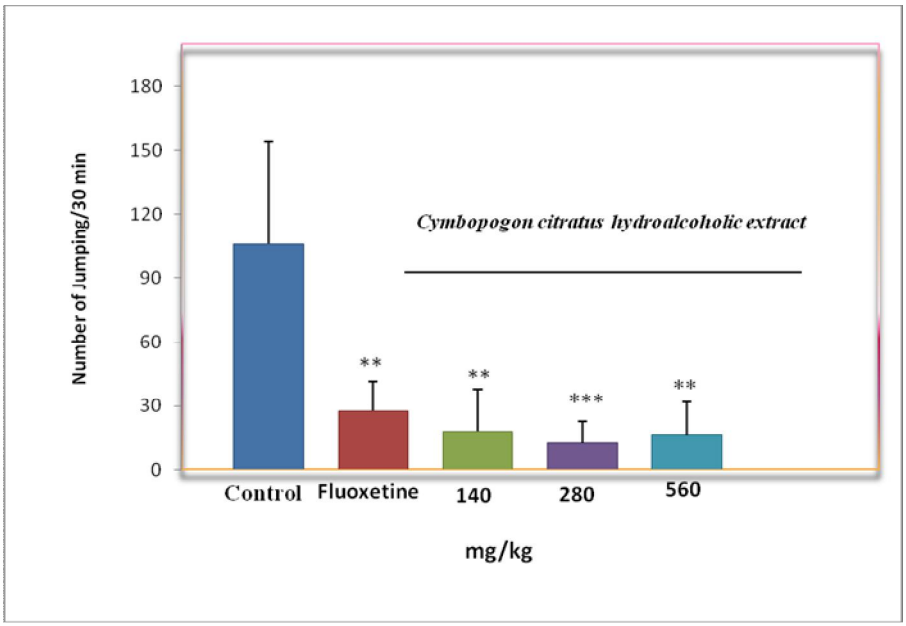

Figure1. Effects of different doses of hydroalcoholic extract of*Cymbopogon citratus* on naloxone-precipitated jumping in morphine-dependent mice (n = 8, Mean ± SEM, ** p < 0.01 and *** p < 0.001 compared to control group, Tukey-Karamer test).

Table 1.Effects of Different doses of *Cymbopogon citratus*on checked signs of morphine withdrawal signs. (n = 8, Mean ± SEM, $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to control group, Mann-Whitney U test).

Treatment/Group	Grooming	Teeth chattering	Climbing	Rearing	Wet dog shakes	writhing	Diarrhea
Negative control (Normal saline)	2±(2-3)	2±(2-2)	2±(1-2)	2±(1-3)	1±(1-2)	2±(1-2.5)	3±(2-3)
Positive control (Fluoxetine 10 mg /kg)	0±(0-0.5) **	1±(1-2)	1±(1-1) *	1±(1-1.5)	1±(1-1)	0±(0-0.5) *	1±(0.5-2) **
Cymbopogon citratus 140 mg/kg	0±(0-1) *	1±(1-2)	1±(0-1) *	1±(1-1)	1±(1-2)	1±(0-1) *	1±(0-2) **
Cymbopogon citratus 280mg/kg	0.5±(0-1.75) *	1±(1-1.75)	1±(1-1) *	1±(1-1.75)	1±(0.25-1)	1±(0.5-1.5) *	1±(1-1.75) **
Cymbopogon citratus 560mg/kg	0.5±(0-1.75) *	0.5±(0-1) **	1±(1-1) *	1±(0.5-1) *	0±(0-0.75) *	1±(0.5-2) *	1±(1-1) **

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RESEARCH ARTICLE

Effect of Vermicompost and Nitrogen Fertilizer on Yield and it Components of Barley (Hurdeum Vulgar L.)

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ABSTRACT

It has been broadly accepted that barley performs better than wheat, particularly under unfavorable conditions of the Iran. However, this assumption is not based on rigorous evidence. This study was planned to examine effect of vermicompost and chemical nitrogen fertilizer on barley on yield and it components of barley. The experiment was laid out in a factorial design based on randomized block design with three replications. Treatments were Vericompost in three levels (0, 10, and 20 ton.ha^{-1}) and nitrogen fertilizer in three levels (0, 10, and 20 kg.ha^{-1}). The results showed that effect of nitrogen fertilizer was significant on number of spike per square and number of grain per spike. However, vermicompost had a significant effect on number of spike per square, grain yield and harvest index. The comparison of the mean values showed that 20 kg.ha^{-1} treatment had the highest and the control treatment had the lowest number of spike per square, number of grain per spike and grain yield. However, application of 20 ton.ha^{-1} treatment had the highest number of spike per square, number of grain per spike, grain yield, biobass yield and harvest index and differences with control was significant. This study demonstrate that vermicompost and chemical nitrogen fertilizer successfully manipulate the growth of barley, resulting in beneficial changes in yield and yield components of barley but vermicompost was more successful for this purpose. So we can apply vermicompost instead of chemical nitrogen fertilizer for health production of barley in organic farming system.

Key words: Barley, chemical fertilizer and vermicompost

INTRODUCTION

Barley (Hurdeum vulgar L.) is the main rainfed crop and it grain yield is variable in response to the erratic rainfall (Austin et al., 1998a,b). However, barley is preferred by farmers under low rainfall conditions. In recent years, about two thirds of barley crop has been used for feed, one-third for malting and about 2% directly for food (Baik and Ullrich, 2008). Farmer preferences to assign barley to areas with higher frequency of severe drought are due to the general belief that it performs better than wheat under drought conditions. Grain yield of small grain cereals is determined by two main components, grain number per unit area (grains perm^2) and mean grain weight. During pre-anthesis, the potential grain number per unit area (Fischer, 1985) and potential grain weight (Calderini et al., 2001) are defined. The final grain number per unit area is set immediately after anthesis, while grain filling occurs during the remaining post-anthesis period (Ugarte et al., 2007). Grain yield is usually strongly associated with the number of grains per unit area (Fischer, 1985; Savin and Slafer, 1991). While this association has been extensively reported for a relatively wide range of environments and nutrition.

The use of organic fertilizers, Vermicompost and crop residues in crop production has been a known activity for hundreds, even thousands of years (Lacko-Bartošová et al., 2005), thanks to new varieties, changing climatic conditions, new aims in cultivation (higher yields, better quality, maximum efficiency of production), new technologies of food processing and efforts for nature protection and sustainable agriculture, these „old“ materials and new technologies are nowadays a target of refinement and globally focused attention (Sharpley et al., 2004; Welke, 2005). Vermicomposts are the products of the degradation of organic matter through interactions between earthworms and microorganisms. Vermicomposts are finely divided peat-like materials with high porosity, aeration, drainage, and water-holding capacity and usually contain most nutrients in the available forms such as nitrates, phosphates, exchangeable calcium and soluble potassium (Arancon et al., 2005).The preparation and use of organic manures as a nutrient management may provide a hygiene and useful way of disposal and utilization of waste which would otherwise have created a healthy environment (Indrakumar, 2009). They told that, Frenchbean was most responsive to vermicompost treatment on growth and yield in comparison to farmyard manure, chemical fertilizer and mixed treatments under irrigated condition of Srinagar valley. They also concluded that vermicompost is particularly good for farmers, consumers and ultimately for soil as it can be used as a resource for maximum crop productivity with more financial output in comparison to those chemical fertilisers. Yadav and Vijayakumari (2003) found better yield in vermicompost treatment. Same observation was also reported by Rameshwar (2006). Guu et al. (1995) reported that yield increased with fertilizer and manure application.

Therefore this study was planned to examine effect of Vermicompost and nitrogen fertilizer on barley on yield and it components of barley.

MATERIALS AND MHETODS

Field material and Experimental design

In order to study response of Vermicompost and nitrogen fertilizer on barley Izeh cultivar an experiments was conducted under temperate condition in Khorramabad, Lorestan provience, Iran during 20013–2014. The experiment was laid out in a factorial design based on randomized block design with three replications. Treatments were Vericompost in three levels (0, 10, and 20 ton.ha^{-1}) and nitrogen fertilizer in three levels (0, 10, and 20 kg.ha^{-1}).

It was sown at 250 kg ha^{-1}. There were 4 rows in each plot; rows were 4 m long with 0.2 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 1 m^2 of the two central rows were harvested. Then yield components were calculated as standard methods with using 8 plant. To

determine grain yield and biomass yield, we removed and cleaned all the seeds produced within middle one m^2 of the four central rows in each plot.

Traits

Then grain yield and biomass yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter and quintals per hectare. Replicated samples of clean seed (broken grain and foreign material removed) were sampled randomly and 1000-grain were counted and weighed. The biomass production was measured on 1 square meter from each treatment at maturity stage. The harvest index was accounted for with the following:
HI = (economical yield / biological yield)

Statistical analysis

Data were analyzed with Proc GLM procedure, SAS (SAS Inst., 1997) statistical software.

RESULTS AND CONCLUSION

Number of spike per square: Analysis of variance of results showed that, the effect of nitrogen fertilizer, vermicompost and interaction between them on the number of spike per square was significant (table 1). The comparison of the mean values for N bio-fertilizers on number of spike per square showed that 20 $kg.ha^{-1}$ treatment had the highest (579) and the control treatment had the lowest number of spike per square (419) and difference between them was significant (table 2). For vermicompost treatment application of 20 $ton.ha^{-1}$ treatment had the highest (561) and application of 10 $ton.ha^{-1}$ treatment had the lowest (395) of it (table2).

Number of grain per spike: The results of analysis of variance showed that, the effect of nitrogen fertilizer on the number of grains per spike were significant only (table 1). The comparison of the mean values for nitrogen fertilizer on number of grain per spike for barley showed that application of 20 $kg.ha^{-1}$ nitrogen fertilizer had the highest (30) and the control treatment had the lowest number of grains per spike (16) and difference between them was significant (table 2).

1000 grain weight: The results showed that, the effect of any of treatments was not significant on 1000 grain weight (table 1).

Grain yield: The results showed that, the effect of vermicompost and interaction between vermicompost and nitrogen fertilizer on grain yield was significant at 1% (table 1). The comparison means values for grain yield showed that application of 20 $kg.ha^{-1}$ nitrogen fertilizer had a highest (335 g) per square and the control treatment had the lowest grain yield (289 g) per square and difference between them were significant (table 2). In vermicompost treatment application of 20 $ton.ha^{-1}$ vermicompost had a highest (409 g) per square and the control treatment had the lowest grain yield (252 g) per square and difference between them were significant (table 2).

Biomass yield: results showed that, the effect of any of treatments was not significant on Biomass yield (table 1).

Harvest index (HI): Results of analysis of variance showed that the effect of vermicompost and interaction between vermicompost and nitrogen fertilizer on HI were significant at 5% (table 1). The comparison of the mean values of HI showed that application of 20 $ton.ha^{-1}$ vermicompost had the highest (40%) and control treamment had the lowest HI (26%)(table 2).

The results of this study showed that effect of nitrogen fertilizer was significant on number of spike per square and number of grain per spike only (table 1). Grain yield for treatments and lines was mainly determined by grain number per unit area. Grain yield variability was the result of the potential growing conditions in each location generated by differences in lines and application of nutrient during the vegetative and reproductive stages. This was also reported in other studies (Austin et al., 1998a,b). In the present study application of nitrogen fertilizer increased number of spike per square and number of grain per spike that results to increase in high grain yield and productivity. The results correspond to Kato and Yamagishi (2011) finding that spikes density of wheat varieties were higher in organically managed field than conventional field due to higher pre-anthesis dry matter production.

However vermicompost had a significant effect on number of spike per square, grain yield and harvest index (table 1). The grain yield was significantly higher in nitrogen fertilizer and vermicompost application treatment. With increased in application of nitrogen fertilizer and vermicompost grain yield increased significantly (table 2). Using organic manure and the application of biofertilizers such as vermicompost and nitrogen fixing bacteria has led to a decrease in the use of chemical fertilizers and has provided high quality products free of harmful agrochemicals for human safety (Moradi et al., 2010). According to the present study, vermicompost with high water-holding capacity and proper supply of macro and micro-nutrients (Arancon et al., 2006; Kumawat et al., 2006), has a positive effect on production of barley and subsequently enhanced grain yield. Improved growth, development and yield of plants have previously been reported in the presence of optimal amounts of vermicompost (Singh and Ramesh, 2002; Darzi et al., 2012). These findings are in accordance with the observations of Pandey (2005) on Artemisia pallens, Moradi et al. (2010).

According to the present analysis, vermicompost have increased weight of 1000 seeds by enhancing the rate of photosynthesis and the biomass production (Roy and Singh, 2006) and increase in yield of barley was more than nitrogen fertilizer (table 2) . The present result is in agreement with the report of Darzi et al. (2007) on F. vulgare. 20 $ton.ha^{-1}$ application of vermicompost was more efficient rather that other amount application of it on grain yield and harvest index (table 2). However the results clearly demonstrate the effectiveness of vermicompost in increasing the biomass yield rather than chemical nitrogen fertilizer (table 2). Vermicompost increases the growth rate because of the water and mineral uptake such as; nitrogen and phosphorus (Zaller, 2007), which leads to the biomass and grain yield improvement. In final we concluded that application of vermicompost increased yield and yield components of barley rather than chemical nitrogen fertilizer. It is clear from the present study that vermicompost and nitrogen fertilizer successfully manipulate the growth of barley, resulting in beneficial changes in yield and yield components of barley but vermicompost was more successful for this purpose. So we can apply vermicompost instead of chemical nitrogen fertilizer for health production of barley in organic farming system.

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Table1. Analysis of variance for yield and yield components of under application of vermicompost and nitrogen fertilizer

S.O.V	DF	Number of spike per square	Number of grain per spike	1000grain weight	Grain yield	Biomass yield	HI
R	2	3342.81	50.70	204.12	11475.65	168613.48	113.34
Nitrogen fertilizer	2	60103.70**	476.50**	78.40ns	5702.65ns	2111.26ns	98.92ns
Vermi compost	2	66059.26**	31.69ns	11.68ns	57038.55**	61102.37ns	345.45*
N*V	4	14561.48**	28.13ns	68.42ns	31322.88**	28642.81ns	236.04*
Error	16	3072.2	31.61	63.51ns	3835.88	23140.8	68.46
CV%		11.31	24.87	17.65	19.46	16.14	24.18

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

Table 2. Mean comparisons for yield and yield components of under application of vermicompost and nitrogen fertilizer

Tretaments	Number of spike per square	Number of grain per square	1000grain weigth (g)	Grain yield ($g.m^{-2}$)	Biomass yield ($g.m^{-2}$)	(%)HI
Nitrogen fertilizer						
0	419b	16b	45	289b	929	30
10 $kg.ha^{-1}$	470b	20b	42	330a	938	35
20 $kg.ha^{-1}$	579a	30a	48	335a	959	36
vermicompost						
0	512a	22	43	252b	911b	24b
10 $ton.ha^{-1}$	395b	20	45	295b	880b	34ab
20 $ton.ha^{-1}$	561a	24	45	406a	1036a	40a

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

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RESEARCH ARTICLE

Effects of Silver and Diet Form on Growth Performance, Carcass Characteristics and Some Serum Enzymes of Broilers

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ABSTRACT

The objective of this investigation was to evaluate the effects of silver (in the form of nitrate) and diet form (pellet or mash) on growth performance, carcass charactristics and some serum enzymes of broilers, in a 2 × 2 factorial design. The first factor was diet form (mash or pellet) and the second was silver nitrate (with or without). Main effects of diet form on daily weight gain, feed intak, feed conversion ratio and final weight were significant ($p \leq 0.05$) but there were interaction effects in total feed intak and feed conversion ratio($p \leq 0.05$). Interactin effects of two factors on carcass percentage and fat percentage were significant. Diet form had significant effect on breast percentage ($p \leq 0.01$). There were no significant effects of treatments on aspartate aminotransferase (AST) and alanine transaminase (ALT) in 21 and 42 days of age and for glucose in 21 day of age. Alkaline phosphatase (ALP) in 21 day of age were significant ($p \leq 0.01$). Main effect of silver nitrate on ALP was significant ($p \leq 0.05$). Based on the results of this investigation, pellet had suitable effects on broiler growth performance and it seems that silver nitrate in this case had negative effects on some serum enzyme such as ALP and must be used in lower concentration in the broiler's diet.

Key words: Silver, Diet form, Broiler performancs, Serum enzymes

INTRODUCTION

Todays, animals, especially poultry, are very sensitive to pathogens [1]. Antimicrobials such as antibiotics are often used to decrease or eliminate harmful microorganisms in the intestine, and to develop weight gain and feed efficiency [2]. It has been demonstrated that the continuous usage of antibiotics as promoters of growth encourage the retention in animal tissues and that the human intake of such animal yealds would potentially progress processes of antibiotic resistance, movements of social pressure towards food security were claiming for a strict control and against their usage in animal nutrition, reaching the banning of using antibiotics as promoters from 2006 in the European Community (CE 1831/2003). Therefore, because of the public concern over possible antibiotic residual effects and the development of drug resistant bacteria, antibiotic using as routine feedstuff additives has been banned in the recent years [3]. This feild has led to the applying of non-antibiotics' substances [4, 10, 13 & 25] and among the alternatives are silver compounds in the forms of ionic and nanoparticles [5, 9, 11, 21, 22, 23 & 24] and hydrogen peroxide [17 & 18].

Silver compounds have been historically applied to inhibit microbial growth [6]. The inhibitory effect of ionic silver is due to several biological events such as attachment to cell membranes, its adsorption to the negatively charged bacterial cell wall, changes of membrane permeability, generating reactive oxygen species (ROS) and de-activating cellular enzymes [7] that hydrogen peroxide can encaurage this effect [18].

Silver has been used as salts (ionic form), mainly nitrate, sulphate or chloride, traditionally. However, silver cation, is converted into the less effective silver chloride in the stomach or bloodstream, and can make complexes with various ligands. Unstable silver nitrate can be toxic to tissues [7]. These features may be having harmfull effects on health, and resulting to high toxicity of silver [8]. Then, the goal of this experiment was to evaluate the effects of silver nitrate and hydrogen peroxide in pellet or mash diet on growth performance, carcass charactristics and some serum enzymes of broilers.

MATERIALS AND METHODS

Management of broilers and experimental design

One hundred and sixty Ross unsexed day old 308 strain broiler chicks were purchased from a commercial hatchery and were randomly divided into four groups with four replicates (1.5 m × 1.0 m, floor pen) ineach treatment on equal body weight (42±0.3 g). Broiler chicks were nourished corn soybean meal based diet (with sunflower oil) as per standards of nutritional requirement recommended by NRC, 1994. Ingredients and chemical composition of basal diet were shown in Table 1. All factors such as temperature, light, water, ventilation and vaccination were similar for all treatments and based on local veterinary office.

In a 2 × 2 factorial design (based on completely randomized design (CRD)), the effect of silver nitrate and hydrogen peroxide (Nanosil®) in broiler feed on growth performance, carcass charactristics and some serum enzymes of broilers were investigated. The first factor was feed type (pellet or mash) and the second was silver nitrate and hydrogen peroxide (with or without). Broilers and feeds were weighed weekly. Feed conversion ratio was calculated by dividing feed consumed to weight gain in each period.

Feed preparation

Tow litters of Nanosil® solution stock (from Kimia Faam Co, Ltd. Iran, contain 500 ppm ionic silver as silver nitrate and 50% H_2O_2) were dissolved in 98 litters of water and 20 litters of later solution mixed with 1 ton of mash diet. Next, this mash diet was pelleted or not. This process applied to starter and grower diet.

Biochemical analysis

At days of 21 and 42, all birds from each replicate were selected for blood samples' collection for estimatingthe serum AST, ALT, ALP and glucose. After blood collection, samples were placed in a room temperature and then serum was separated and centrifuged at 3000 rpm for 15 min in centrifuge machine. Serum samples were frozen and stored at -20°C, and analyzed subsequently. Serum metabolites analyzed by using appropriate laboratory kits (Pars Azmon Co, Ltd. Tehran, Iran).

Carcass charactristics

At 42 days of age, four birds per replicate (2 male and 2 female),were slaughtered and breast, thight and fat were separated and weighed.

Statistical analysis

All data were analyzed using GLM model of SAS [20] (SAS Institute Inc, 1996) for analysis of variance. Significant differences among the treatments were identified at 5% level by Duncan's multiple range tests.

RESULTS

Performance

In Table 2 and 3, the effects of treatments on weight gain, feed intake and feed conversion rate and live body weight were shown. Main effects of diet form on daily weight gain, feed intak, feed conversion ratio and final weight were significant ($p \leq 0.05$) but there were interaction effects in total feed intak and feed conversion ratio($p \leq 0.05$). Main effects of Nanosil on weight gain, in days of 1-42, were significant ($p \leq 0.05$).

Interaction effects of feed type and silver as Nanosil on feed intak, in days of 1-42, were significant ($p \leq 0.05$). Interaction effect of feed type and Nanosil on feed conversion ratio in total period (days of 1-42), was significant ($p \leq 0.05$). Main effects of feed type on feed conversion ratio, in days of 1-42, were significant ($p \leq 0.05$).

Carcass charactristics

In Table 4, the effects of treatments on carcass charactristics were shown. Interactin effects of two factors on carcass percentage and fat percentage were significant. Diet form had significant effect on breast percentage ($p \leq 0.01$).

Blood parameters

Data of blood parameters were shown in Table 5. There were no significant effects of treatments on AST and ALT in 21 and 42 days of age and for glucose in 21 day of age. Interactin effects of factors on glucose (in 42 day of age) and

ALP (in 21 day of age) were significant ($p \leq 0.01$). Main effect of silver nitrate and hydrogen peroxide on ALP was significant ($p \leq 0.05$).

DISCUSSION

Advantages of pellet diet in broiler nutrition were discussed in previous investigations [15 & 19]. Contrary to the findings of the present study, Ahmadi (2009) and Ahmadi and Kurdestani (2011) reported that silver in the form of nanoparticles had no effect on weight gain of broilers. However, in our investigation, ionic silver was used. Birds consume more feed in pellet form than mash form [15 & 19]. Broilers consuming pellet diet with Nanosil had better feed conversion ratio than other treatments and interaction was significant. Probably, this action is due to the effect of ionic silver on harmful bacteria in intestine and resulted in healthy gut and better absorption of nutrients in broilers receiving pellet diet with Nanosil. It seems that all positive effects of silver on performance is related to recent action on gut microflora [14 & 26] that encourages with H_2O_2 [12, 17 & 18].

Absorbed silver ions from gastrointestinal tract enter liver through the portal vein and might have impact on the liver since the liver serves as the first checkpoint for everything absorbed before becoming systemic. Liver is able to actively remove compounds from the blood and transform them to chemical forms that can easily be excreted. It is a logical assumption that ingested silver nano-particles might have impact on the liver. Significant amounts of silver in the liver were observed after inhalation [10]. Its renal excretion via the urine has been detected [23]. The results of researches showed that silver (especially in the form of nano-particles) can damage to different organs and tissue such as liver cells [6 & 23]. Increasing level of ALP in serum indicate cell damage.

In conclusion, based on the findings of this investigation diet of broiler chicks in the form of pellet had better effects on performance than mash diet and the concentration of silver with applying Nanosil in the broiler diet have to decreases.

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Table 1. Composition of experimental diets (% diet).

Ingredient	starter (0-21)	grower (22-42)
Corn	59.17	63.81
Soybean meal	35.97	30.24
Sunflower oil	1.25	2.5
DCP	1.42	1.38
dl-methionine	0.11	0.07
Oyster	1.26	1.25
Salt	0.32	0.25
Vitamin/mineral premix ≠	0.5	0.5
Calculated composition ≠≠		
ME (kcal/kg diet)	2900	3000
CP	20.84	18.52
CF	2.7	2.3
Ca	0.9	0.88
P (available)	0.41	0.4
Na	0.18	0.18
Lys	1.12	1.10
Met+Cys	0.81	0.75
Analyzed coposition		
CP	20.52	18.33
Ca	0.92	0.89
P (total)	0.52	0.49

≠ Provided per kg of diet: vitamin A, 8000 IU; cholecalciferol, 2000 ICU; vitamin E, 30 mg; manadione, 2 mg; riboflavin, 5.5 mg; pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; vitamin B12, 0.02 mg; folic acid, 0.5 mg; thiamin, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg; ethoxiquin, 125 mg; Mn, 65 mg; Fe, 55 mg; Cu, 6 mg; Zn; 55 mg.

≠≠ Based on NRC, 1994.

Table 2. Effects of silver nitrate and H_2O_2 (Nanosil) in pellet or mash diet of broilers on daily weight gain, feed intake and feed conversion ratio in different period.

Trait	Diet form					ANOVA (probability)		
	Pellet	Pellet	Mash	Mash				
	Silver (Nanosil)					Diet Form	Nanosil	Diet Form* Nanosil
	+	-	+	-	SEM			
Weight Gain, (g)								
Days 1-21	28.20	26.31	27.52	25.81	0.91	ns	ns	ns
Days 22-42	83.33[a]	77.95[a]	52.98[b]	52.97[b]	2.71	**	ns	ns

Days 1-42	55.76[a]	52.13[b]	40.25[c]	39.39[c]	0.92	**	*	ns
Feed Intake, (g)								
Days 1-21	35.06	34.83	34.49	33.56	0.47	ns	ns	ns
Days 22-42	134.38[a]	134.88[a]	133.00[a]	127.50[b]	1.43	*	ns	ns
Days 1-42	84.72[a]	84.86[a]	83.75[a]	80.52[b]	0.66	**	*	*
Feed Conversion Ratio, (g/g)								
Days 1-21	1.24	1.33	1.25	1.31	0.046	ns	ns	ns
Days 22-42	1.61[a]	1.74[a]	2.51[b]	2.41[b]	0.04	**	ns	ns
Days 1-42	1.51[c]	1.62[b]	2.08[a]	2.04[a]	0.025	**	ns	*

[a-c:] Means in each row with different superscript are significant different ($p \leq 0.05$).
ns: not significant.

Table 3. Effects of silver nitrate and H_2O_2 (Nanosil) in pellet or mash diet of broilers on live weight in different ages.

Live weight, (g)	Diet Form					ANOVA (probability)		
	Pellet	Pellet	Mash	Mash				
	Silver (Nanosil)					Diet Form	Nanosil	Diet Form* Nanosil
	+	-	+	-	SEM			
Day 7	155[ab]	163[a]	139[bc]	131[c]	5.1	**	ns	ns
Day 14	369[a]	373[a]	313[b]	314[b]	11.8	**	ns	ns
Day 21	631	595	623	588	20.9	ns	ns	ns
Day 28	828	802	799	775	12.3	ns	ns	ns
Day 35	1572[a]	1510[b]	1427[c]	1397[c]	17.7	**	*	ns
Day 42	2371[a]	2232[b]	1728[c]	1696[c]	39.4	**	ns	ns

[a-c:] Means in each row with different superscript are significant different ($p \leq 0.05$).
ns: not significant.

Table 4. Effects of silver nitrate and H_2O_2 (Nanosil) in pellet or mash diet of broilers on carcass charactristics.

Trait	Diet Form					ANOVA (probability)
	Pellet	Pellet	Mash	Mash		

	Silver (Nanosil)				SEM	Diet Form	Nanosil	Diet Form* Nanosil
	+	-	+	-				
Carcass weight, (g)	1575.5[a]	1479.1[b]	1241.6[c]	1189.4[d]	36.4	**	**	ns
Carcass, (%)	66.4[c]	66.2[c]	71.8[a]	70.1[b]	0.64	**	**	*
Breast, (%carcass)	30.8[b]	32.0[b]	33.1[a]	33.5[a]	1.0	**	ns	ns
Thight, (%carcass)	22.7	23.1	22.9	22.6	0.85	ns	ns	ns
Fat, (%carcass)	1.49[b]	1.23[c]	1.47[b]	2.00[a]	0.08	**	*	**

[a-d:] Means in each row with different superscript are significant different ($p \leq 0.05$).
ns: not significant.

Table 5. Effects of silver nitrate and H_2O_2 (Nanosil) in pellet or mash diet of broilers on some serum enzymes and glucose.

Metabolite	Diet Form					ANOVA (probability)		
	Pellet	Pellet	Mash	Mash				
	Silver (Nanosil)					Diet Form	Nanosil	Diet Form* Nanosil
	+	-	+	-	SEM			
Day 21								
AST, (IU/L)	191	190	218	212	11.6	ns	ns	ns
ALT, (IU/L)	3.0	3.5	3.7	4.0	0.75	ns	ns	ns
ALP, (IU/L)	11951[a]	8299[b]	8162[b]	7916[b]	408	**	**	**
Glucose, (mg/dl)	149	144	143	145	1.6	ns	ns	ns
Day 42								
AST, (IU/L)	200	249	185	227	23.0	ns	ns	ns
ALT, (IU/L)	5.0	3.2	1.8	2.7	2.0	ns	ns	ns
ALP, (IU/L)	4947[a]	2562[b]	4620[a]	3837[ab]	583	ns	*	ns
Glucose, (mg/dl)	142[b]	162[a]	145[b]	143[b]	3.3	*	*	**

[a-b:] Means in each row with different superscript are significant different ($p \leq 0.05$).
ns: not significant.

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RESEARCH ARTICLE

Morphology, Anatomy and Compare Trichometypes of the Medicinal Plant Salvia Pachystachys in the North East Iran

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ABSTRACT

In this study morphological and anatomical structure of leaves, petioles and stems of Salviapachystachys belongs to the mint family (Lamiaceae) in North Khorasan province were investigated. So, after collecting, cutting and coloring, the plant in terms of internal structure, such as the number of layers of the epidermis, coleanchyma, parenchyma, the form of resistance tissue on the vascular bundles, the form of the main vein, the arrangement of mesophyll and number of layers of spongy and palisade parenchyma also been studied,also size of studied parts were expressed in micrometersand general shape of main veins elliptic-circular and petioles was triangular.Isobiolateral mesophyll and 2-4 layers of spongy parenchyma, there was in the center which with 2 layers of palisade parenchyma was on two sides surrounded,In comparison of trichome kinds at plant level, the highest density of non-glandulartrichome on leaves and lowest density were found in the stems, sickle, multicellular, peltate and Capitatetrichomes at plant epidermis was seen.

Key words: Morphological, Anatomical, Salvia pachystachys, Lamiaceae

INTRODUCTION

Mint family in a global distribution contains 7200 species belonging to 236 genera is the economic importance of medicine (Buyukartalet al., 2011). Salvia is the largest genus in Lamiaceae family , there are about 1000 species of tropical and temperate widely present, most of sage in 3 distinct areas.there are 500 species in Central and South America, 200 species West Asia and East Asia , 100 species (walker and sytsma, 2007). Salvia species are used worldwide as a traditional medicine, anti-bacterial properties, antioxidant and anti-tumor are properties of the plant.

Many species of this genus as tea, food, cosmetics and perfumery used (Kahramanetal., 2010). The fatty acid composition of 25 species reported in the sage, is palmitic acid, oleic acid, linoleic acid and linolenic acid (Kiliket al., 2007). In past times, the leaves of this plant are collected, dried and burned to repel insects (Mayekisoet al., 2009).trichomes as appendages, unicellular or multicellular which are created from epidermal cells, can be found on all parts of the plant development,Trichomes of plants can vary in structure and function and taxonomically is importance (Coisin and Gostin, 2011).Mousavi and his colleagues in 2014, micromorphological-anatomical study of the leaves of some species of Salvia in the North East have done (Mousaviet al., 2014). Morphologic, anatomical Karyological assessment SalviaSclareaby Özdemir and Senel was done. (Ozdemir and Senel, 1999).Shirsat and colleagues in 2012 Morphological, anatomical study of Salvia Plebeia conducted in India(Shirsatet al., 2012).Özdemir and colleagues in 2009AnatomySalviaviridis have studied (Ozdemiret al., 2009). Bercu and colleagues the anatomical and histoAnatomy studyofspecies Salvia Nutans performed(Bercuet al., 2011).Since the study and research on this material is limited, and the of morphological, anatomical study, and especially to study and compare the trichomes in the plant Salviapachystachysnot been done,So research on this species was necessary, in order to specify change its internal structure and the types of the trichomes.

MATERIAL AND METHODS

The study of the morphology, number of samples of the species Salvia pachystachys in North Khorasan Province, West Bojnoord, protected area Ghorkhod in April to July intervals year were collected And in addition to new study, some herbarium specimens Institute of Plant Sciences, Ferdowsi University of Mashhad (FUMH) with herbarium number 44,476 is also assessed. And various sectors such as leaf shape, bracelet, the trichomes density on leaves, petioles and stems, inflorescence shape, shaped calyx, surface pedicellus, the outer and inner surface of the corolla, etc. were studied.

The study of the Anatomythe matured dry leaves, stems and petiole of S. pachystachyspreserved in Formalin: Acetic acid: Alcohol ethylic (FAA) solution was selected. Then, manual cross sections were prepared and by using green methyl and carmine stained. Then, the sections were photographed by different magnifications of light microscope Labomed model CZ500 and Dino capture camera (Mousaviet al., 2014).In this section, the anatomical characteristics such as epidermis, Collenchyma , parenchyma, the resistance tissue (sclereid) shape of the vascular bundles, the main vein, Xylem and Phloem, types of glandular and non-glandulartrichomes, makeup mesophyllic type, number of layers of spongy and palisade parenchyma also was investigated.

RESULTS

Morphological Study

Salvia pachystachys perennial plant, bush, stem length of 30 -50 cm, lying partly standing, green, hard, thick and woody basal part wood, the lower part is covered with short hairs, upper part of covered with glandulartrichomespeltateand capitate type.Petiole 2-5 cm of glandularthe andnon-glandulartrichomes. Oblong to oblong - linear, pinnatisect leaves, scattered throughout the stem, covered with short, soft hairs and non-glandular and glandulartrichomesveryand dense, hairy dorsal surface, serrated leaf margins, raceme-cyme inflorescence, cycles with intervals of 6-2 flowers,Bracts length 16-18 and width of 6-10 mm, Oval, flat, the trichomes in the front and back short Bracts, Trochus, white and purple. Pedicel 6-3 mm long, less the trichomes, Calyx at the time of flowering 12 cm and fruiting 16 mm, Glassy, green, veiny, with glandulartrichomes and the often long and non-glandulartrichomesand warty glandulartrichomes, Upper lobe short, curved, three-toothed and thorny, middle tooth larger than the lateral, corolla 20-35 mm long, almost white and the inner part is pale violet, right corolla tube, a the trichomes ring, stamens 2 pcs ,sterile anthers, nutlet fruit, with a length of 1/93 and 1/90 mm wide, round, brown.

Anatomical study

Leaf: The study of Salvia pachystachisleaves transect showed thatmidrib was, elliptical – ovalwith prominent upper surface, 1-2 layers of the epidermis Square, 3- 4 coleanchyma layer surrounding the midrib, 5-8 polygonal parenchymal cells layer after layer lower epidermis, sclerid almost a whole bunch in the vascular bundle presents,Mesophyllic leaves of isobiolateral, 2-4 layers of spongy parenchyma there was a in the center with 2 layers of palisade parenchyma on two sides was surrounded, non-glandulartrichomes the with mass density and glandulartrichome was lower (Fig. 1. A, B, C, D-Table 1).

Petiole: Petioles triangular shape, the edges are slightly raised, slightly recessed upper part and lower part was slightly raised. 1 layer of the Square epidermis , 2 layer coleanchyma, 6-9 Layer polygonals parenchymal cells of the after lower epidermis layer was observed, sclerid completely separate on vascular bundle present. Non-glandulartrichomes are thicker and less dense hairs and number ofglandulartrichomes the was higher (**Fig. 2. A, B**-Table 1).

Stem: Salvia species stems from the structure does not show much difference.But distinction stems Salvia species can specify the types of the trichomes. Stem in this species, with a thin layer of the cuticule and the subcuticule, a square or rectangular layer of epidermis present. at the corners, 4-8 coleanchyma layer was observed. 6-8 parenchyma layer below coleanchyma. Sclerid tissue as a whole class, a vascular bundle surrounded. 28-34 layers pith parenchyma was present. There are scattered glandular and non glandulartrichomes (**Fig . 2 C -**Table 1**).**

Study trichomes in the surface of leaves , petioles and stems of the plant Salvia pachystachys

Types of glandular and non-glandulartrichomes on the plant epidermis, which several trichomes types of Salvia species has been mentioned Including: glandulartrichome with a very long base -mesophyll leaves(A), very short single-cell basis - mesophyll and midrib of leave and petiole (B) two-cell glandulartrichomes as primary cells are much longer than the end of the cell. Mesophyll and midrib of leaves, petioles and stems (C) glandulartrichome with three cells, so that intermediate cells are larger than two other cell. The midrib of leaves and petioles (D).peltateglandulartrichomes, mesophyll and midrib of leave (EFG).

In a general rule, the number of non-glandular trichome of S. pachystachys the epidermis so much more than the glandulartrichomes,Including types of trichome on leave, petiole and stem of the plant are as follows single-cell non glandulartrichome with a high length - mesophyll leaves (H), conical non glandulartrichomes very short- mesophyll leaves and stem (I). two cell trichome which apical cell with very high length, and primary cell is very short-Mesophyll leaves, petiole and stem (J, K). nonglandulartrichome with 2 cells with end cell wider than the primary cell - Mesophyll leaves (L). nonglandular trichome with geniculate final cells - mesophyllleaves (M). Trichome 3-cell with slow end (semicircular) - midrib leaves (N). Non glandular Trichome on the petiole is much thicker than the glandularTrichomes (O).Trichomesickle 7 cell that is rarely seen - mesophyll leaves. (P). Hairline trichomeswith apical cell very long - petiole (Q).

DISCUSSION

This study aimed to provide information about the morphology and anatomy of the species S.pachystachys.This is the first report about the anatomical cases and comparetrichomes types in different sections of this species.As mentioned, shape of midrib in this species is elliptical – oval and higher density of non-glandulartrichomes in leaf were most evident and petiole epidermis have higher glandulartrichome and the mesophyll was isobiolateral. Mousavi and colleagues in 2014, leaf anatomy some species Salvia of in Iran were investigated. And showed that the species Salvia atropatana have a elliptical-circular midrib.Peltateglandulartrichomes and non- glandulartrichomes 4-5

cells were observed on the leaf surface. And for these species, 2 layers coleanchym and 7 layers of parenchyma, crescent-shaped handle on top of a bunch of vascular sclerid, mesophyllicdorsi ventral reported(Mousaviet al., 2014). In the present study, we found several types of trichomes, as the triangular petioles from sides is raised. Completely separate sclerid bundle at the top of a vascular bundle is present. Stem has a square or rectangle layer of the epidermis.andsclerid texture as complete set, surrounds the vascular bundle. Metcalfe and Chalk stated that the arrangement of the vascular bundle in the petiole of the mint family is important in terms of taxonomy(Metcalf and Chalk, 1972). Ozdemirand colleagues S. viridisdetermined that the petiole, epiderm from cell layer Elliptical- circle is formed. Parenchyma consists of 5-10 layers of parenchyma cells with intercellular spaces was clear(Ozdemiret al., 2009).Ozdemir and Senel in reported that leaves of S. sclarea have 2-3 layers of palisade parenchyma, cells of regular coleanchym, and non -glandular and glandulartrichomes on the upper and lower part of the epidermis, more trichomes are Unicellular or multicellular glandular.and in the petioles, more trichomes are glandular type. On the surface of stems, there are glandular and non-glandulartrichomes andon the surface of stems, there are glandular and non-glandulartrichomesandand most of the trichomes is glandular(Ozdemir and Senel, 1999).Shirsat and colleagues in 2012 stated that S. Plebeia have a variety of glandular and non-glandulartrichomes instems, leaves, petioles, calyx and Corolla.Capitatetrichomes greatly in structure, size, located in parts of the plant and the secretion status are different(Shirsatet al., 2012).Peltatetrichomes family lamiaceae, including several glandular cells between 8-16 cell of the in the head. and has a short stem and base epidermal cells (Hallahan, 2000).Bercu and colleagues in 2011 reported that S. nutans has both glandulartrichomesPeltate, Capitate, and also non-glandulartrichomes multicellular up to 4 cells (Bercuet al., 2011).

CONCLUSION

In addition to morphology, features such as the shape of the midrib of leaves, petioles, and how the sclerid on vascular bundle and especially those associated with glandulartrichomesPeltate and Capitate, and non-glandulartrichomes cell in identifying species S.pachystachys very effective.

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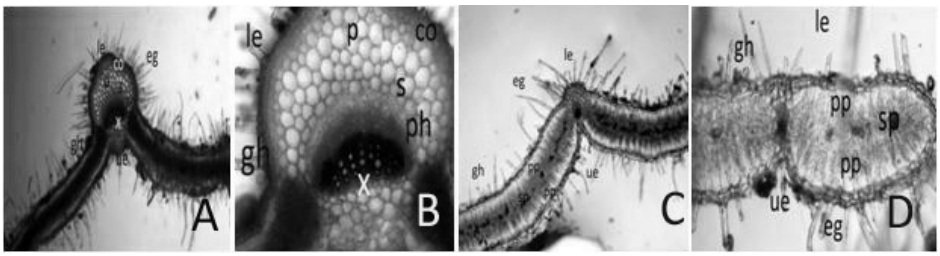

Fig . 1. Cross sections of Salvia pachystachys leaves (× 40, 100).(A, B,C, D).Ue: upper epidermis; Le: lower epidermis; Co: collenchyma; Pc: parenchymatic cell; S: sclerenchyma; X: xylem; Ph: phloem; Pp: palisade parenchyma; Sp: Spongy parenchyma

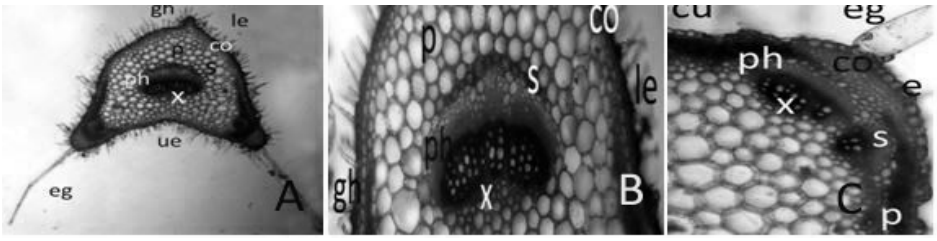

Fig . 2.Cross sections of Salvia pachystachys petiole and Stem (× 40, 100).Ue: upper epidermis; Le: lower epidermis; Co: collenchyma; P: parenchymatic cell; S: sclerenchyma; X: xylem; Ph: phloem; cu: cuticule; e: epidermis; p: parenchyma; Pr: pith parenchyma

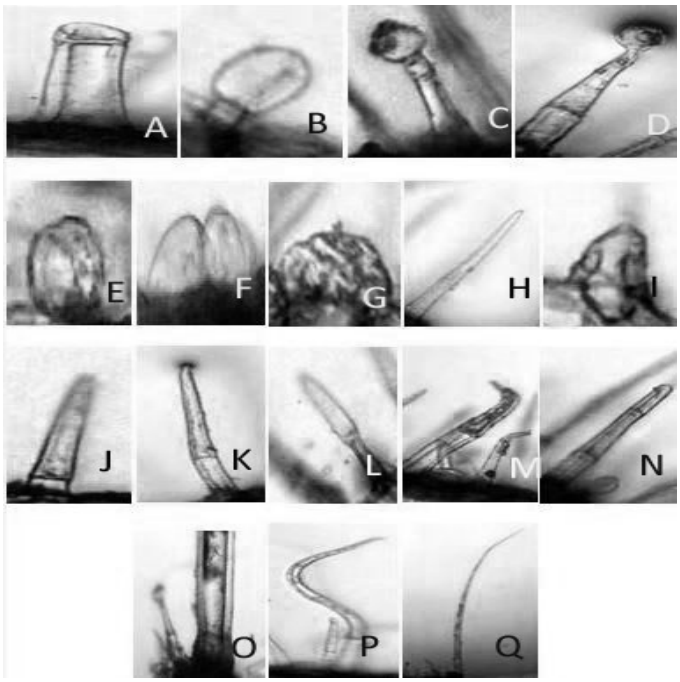

Fig3: types of trichomes on leaves, petioles and stems of the plant S.pachystachys

Table 1: Anatomical measurements of plant organs of Salvia pachystachys.

Leaf Anatomy	Length (μm) Min-Max	**Petiol Anatomy**	Length (μm) Min-Max	**Stem Anatomy**	Length (μm) Min-Max
upper epidermis	10/77-22/03	upper epidermis	10/88-19/11	cuticule	1/72-2/20
lower epidermis	12/00-31/15	lower epidermis	11/90-24/16	epidermis	13/43-19/00
collenchyma	14/18-46/23	collenchyma	15/14-39/05	collenchyma	17/14-45/70
parenchymatic cell	28/55-51/13	parenchymatic cell	27/17-55/90	parenchyma	30/67-51/43
sclerenchyma	13/72-38/17	sclerenchyma	12/70-31/49	sclerenchyma	18/02-38/60
xylem	20/45-49/01	xylem	22/40-39/81	xylem	25/89-48/80
phloem	10/15-34/80	phloem	11/50-28/13	phloem	11/25-29/16
palisade parenchyma	14/30-26/55				
Spongy parenchyma	13/80-21/09				

RESEARCH ARTICLE

The Effect of PMSGInjection (Various Doses) at CIDR Removal on Ovarian Follicular Activity and Reproductive Performance in GhezelEwes

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ABSTRACT

According to the importance of lambing rate to profitability of sheep holders, this trial investigated the effects of various doses of pregnant mare serum gonadotropin (PMSG) injection at controlled intravaginal drug-releasing device (CIDR) removal on multiple births in synchronized Ghezel ewes. 137 cycling, multiparous fat-tailed Iranian Ghezel eweswere used in the trial.All animals were divided randomly into four groups then a single intramuscular (IM) injection of PMSG (group 1, 350 IU, n=40; group 2, 450 IU, n=40; group 3, 550 IU,n= 40), group 4 (n=17) was made apart from 1 ml normal saline solution which was used as control group at time of CIDR removal.The estrous cycles were synchronized using CIDR's inserted for a period of 14 days and ewes were mated with the proven rams. Ovarian follicular activity was monitored with the aid of transrectal ultrasonography: on the day of CIDR removal and a day later (at estrus). Pregnancy was diagnosed with the aid of transabdominal ultrasonography 30 days after mating. Following PMSG treatment, the mean number of large follicles on both ovaries increased in the 550 IU PMSG group vs. control group (1.11±0.90 vs. 1.63±0.78) ($P < 0.05$). Lambing rate was 67.5%, 77.5%, 77.5% and 58.8% in groups 1, 2, 3 and the control group respectively. The results show that PMSG injection at CIDR removal, caused large follicles development, but nosignificant difference was seen

between the four treatment lambing rates and also, the lambing rate increased in groups treated with PMSG.

Key words: Pregnant mare serum gonadotropin (PMSG), Controlled Intravaginal Drug-Releasing (CIDR), Ghezel ewes, Ovarian Follicular Activity, lambing.

INTRODUCTION

The most economically important trait in sheep production is the reproduction and it can be manipulated using hormonal treatments [7]. There are several methods for improving reproduction in ewes. One of the way is toincrease the proportion of multiple-ovulated ewes in order to improve lambing rate [2]. Exogenous hormones and synchronization methods are used to maintain and increase lamb production all around the year. One injection of PMSG can stimulate follicular growth, and higher ovulation rate in outside the breeding season [12]. Gonadotropins such as PMSG [11], follicle-stimulating hormone (FSH) [9] and gonadotropin-releasing hormone (GnRH) [1] treatment increase the number of follicles, ovulation rate and litter size. PMSG and progesterone analogues are used vastly to control fertility in cyclic and anestrous ewes, and to synchronize estrus for fixed-time artificial insemination.When no PMSG was injected during estrus synchronization, ovulations occur later and with a longer period of time [8].Therefore rate of ovulation is affected by the PMSG dose employed. An adequate dose of PMSG improves prolificacy, but the use of a high dose produces multiple gestations and thus, an increase in fetal or lamb mortality. So, to avoid non-desirable fetal losses and large litter sizes, the dose level of such gonadotropin has to be precisely adjusted according to the season of the year and the physiological state of the ewes. When PMSG administration is performed at the time of progestogen sponge removal after 12-16 days (mean 14) the onset of estrus antedates and ovulation rate increases then, it enables ovulations to converge. So far, many studies have been made on progestogen sponges or progesterone sponge + PMSG administration [17].

However, the reactionagainst hormone treatment vary according to breeds, kind, administration time and dosage. Due to several factors abovementioned, different breeds show different reproductive traits [17]. Nowaday, there were no any study concerning different doses of PMSG treatment in Ghezel ewes during breeding season. Therefore, the present study was aimed the effect of different PMSG dose treatment on lambing rate in synchronized Ghezel ewes by using controlled internal drug release device (CIDR) during the breeding season .

MATERIALS AND METHODS

The experiment was conducted during the breeding season (October to February), at breeding station of Ghezel sheep in Miandoab in West Azarbaijan province in Iran.

Synchronization Protocol

137 cycling, multiparous fat-tailed Iranian Ghezel ewes, weighting 45-55 kg, were used in the trial. All animals were divided randomly into four groups then a single intramuscular (IM) injection of PMSG (group 1, 350 IU, n=40; group 2, 450 IU, n=40; group 3, 550 IU, n= 40), group 4 (n=17) was made apart from 1 ml normal saline solution which was used as control group at time of CIDR removal. All animals had not previously been used for any PMSG treatment, superovulation or as embryo recipients in a MOET program. The estrous cycles were synchronized using CIDR's (EAZI-BREED™, CIDR®, NewZealand) inserted for a period of 14 days. Ewes were mated with the proven rams. All animals were provided with water and manually fed alfalfa hay supplemented with grain pellets, ad libitum.

Ultrasonography Studies

Ovarian follicular activity was monitored by transrectal ultrasonography (Piemedical, Falco100; Holland, 8 MHz) (Figure1), at CIDR removal and at estrus. The ultrasonographic scanning of both ovaries was recorded using a MP4 player (Marshal X720, China). In brief, during the ultrasonographic evaluations, ewes were kept in a darkened room and restrained in a fostering crate in the standing position. After introducing a hydro soluble contact gel into the rectum (to enhance the ultrasound transmission), the probe was placed into the rectum with the transducer oriented perpendicularly with the abdominal wall. When the urinary bladder was surpassed and the uterine horns were located, the probe was rotated laterally clockwise for 90° and counter-clockwise for 180° to evaluate both ovaries and their structures. One experienced operator performed all the recordings. The ovaries were scanned in several planes to identify all visible follicles (>1 mm in diameter). All follicles larger than 1 mm were counted and classified according to their diameter in one of the following classes: small (≤2 mm), medium (3 mm) and large (≥ 4mm) follicles. Pregnancy was diagnosed with the aid of transabdominal ultrasonography 30 days after mating.

Statistical Analyses

The number of ovarian follicles (that is, small, medium and large follicles) was analyzed using the mixed procedure of SAS (9.1). Mean comparison was performed by least square mean method. The analysis included sources of variation due to treatment groups, days (repeated measures) and their interactions. The differences were compared by Tukey test. Effects of weight and age of ewes were added as a covariate to the model. The percentage data were analyzed using the chi-square test. Significant differences between treatments were determined at the $P < 0.05$ level. Data were expressed as the mean±SEM, unless otherwise stated.

RESULTS

The follicular responses following four groups of PMSG treatment and control group are shown in Table 1. The results of ultrasonography showed that the mean number of small and medium follicles on the right, left and both (right+left) ovaries between the treatment and control groups was not different ($p>0.05$) at estrus (day 1). No significantdifference ($p>0.05$) was observed in the mean number of medium follicles on the right and both ovaries between the groups (Table 1). Following PMSG treatment, the mean number of large follicles increased in the PMSGgroups vs. control group ($p<0.05$) atestrus (Table 1). All ewes showestrus behavior within 48 to 54 h after CIDR removal, and then four rams were introduced randomly to each group. The ewes were diagnosed pregnant by ultrasonography 30 days after ram induction. After lambing, the lambing rate in 550 IU PMSG group ewes (77.5%) was more than that in eweswho received no PMSG (58.82%), (Table2).

DISCUSSION

In this study, ewes' ovary evaluation was conducted via transrectal ultrasonography and during ovaries detection all ewes had standing position that was more comfortablefor them than dorsal recumbency [14]. According to Dogan and Nur findings [11], PMSG stimulates the number of folliclesand this caused more follicular development; the results are in line with our findings. In this study, PMSG administration at day 0 had desirable effects on ovarian enlargement and responses.Pregnant mare serum gonadotropin (PMSG)is used for induction of ovulation and estrus is used most effectively for synchronization during thebreeding season. In addition, pregnant mare serum gonadotropin (PMSG) has been found to increaseovulation rate and twinning in a dose related manner. Between all endocrine approaches to increasereproductive performance, administration of PMSG is more usual than others. Injection of PMSG at theend of the progestogens treatment causes more precise synchronization of estrus in small ruminants[18].

The results obtained from this experiment showed that in the three groups of PMSG treatment the mean number of small and medium sized follicles on left, right and both ovaries was not significantly different in CIDR removal and estrus days. Ali [3] reported that the injection of 300 IU PMSG, 48 h before or at sponge removal had no effect on ovulation rate and these findings are in agreement with our outcomes.Zonturlu et al. reported that the different PMSG doses (300 IU, 400 IU, and 500 IU of PMSG) did not affect ovarian enlargement and responses when compared to the control group [21].

However, there are many factors influencing the effect of PMSG, including the dose and administration time of PMSG, and season. Similarly, Karagiannidis et al. reported that responses to different PMSG doses varied among various breeds [13].

In our observations the mean number of large sized follicles at estrus day on the left ovary was more than that of the right ovary, and it was significantly different ($P<0.05$) in the PMSG administration groups vs. control group (Figure2). We can attribute it to the effect of PMSG on heightening the size of antral follicles but the mean number of large sized follicles on the right ovary, at estrus day was not different significantly ($P>0.05$); that could be due to the same effect of PMSG administrations on ovulation rate. The average number of total large follicles in PMSG treatment groups increased significantly ($P<0.05$) at estrus. Ourfindings are in agreement with that of Cline et al. [10].Also, the effect of PMSG administration dosage can beaffected by variation in breeds genetic and differentbreeds have variable responses [3].

In the present study, during the breeding season, Ghezel ewes were administered different doses of PMSG following a 14-day progesterone treatment. This protocol produced similar results in groups 1, 2 and 3 (95%, 92.5% and 97.5%), which were higher than those obtained in the control group. Luther et al. reported estrus rates of 86.7% and 75% in sheep, which were administered 400 IU of eCG after the administration of implants for 14 days and sponges for 12 days, during the breeding season [16]. Ataman et al. reported to have obtained an estrus rate of 100% during the breeding season with injections of PGF2α and 400 IU PMSG after long and short term progesterone treatment, and an estrus rate of 80% outside the breeding season[1], Ataman et al. obtained significant difference in pregnancy and estrus response for FGA-30 than those of groups[9]. Furthermore, Aköz et al. reported to have obtained an estrus rate of 100% with PMSG injections of 300 and 700 IU, and estrus rates of 93.3% and 100% with injections of 40 mg FGA after a 7-day treatment with 30 mg of progesterone [2]. They reported that the administration of 700 IU of PMSG was more effective in ewes outside the breeding season. Differences in the results obtained are considered to be related to the time of administration, duration of progesterone administration and climatic conditions.

In the present study, pregnancy and lambing rates were similar to between found to be the treatment groups and compared to the control group, but these results were not statistically significant between treatment and control group. Aköz et al. observed no significant difference in pregnancy rates for different PMSG doses (300 IU, 500 IU and 700 IU), [2].Koyuncu et al. reported that the administration of 700 IU of PMSG increased multiple-birth rates and lambing rates[15].Zeleke et al. recorded a pregnancy rate of 75% and a lambing rate of 94.6% in ewes treated with sponges and 300 IU of PMSG [20].

Ewes' lambing rates in this study were affected by dose of PMSG treatment (Table 2).

Thelambing rates were 67.5%, 77.5%, 77.5% and 58.82% in groups 1, 2, 3 and the control group, respectively. No significant differences in term of the lambing rate were recorded between groups in agreement with previous reports [21]. Al-Merestani et al. in a study in which Syrian Awassi sheep were treated with intravaginal sponges combined with 400 IU of PMSG, reported a lambing rate of 78%[4]. It was thought that fertility parameters could be affected by different treatment seasons such as anestrus, breeding or transition season.The observed variation depends on

various factors such as breed, age, time and dose of PMSG administration [11].Salehi et al. (2010) reported that the different sheep breeds have been identified as a major source of variation in the superovulatory response [19].

CONCLUSIONS

In conclusion, using estrus synchronization by progesterone CIDR accompanied with various doses of PMSG injection due to all ewes estrus synchronization, the mean number of large follicles following PMSG treatment increased significantly ($P<0.05$), but the mean lambing rate of the treatment groups was not significantly difference (77.5% vs. 58.82%). Therefore, PMSG administration is effective for the increase of ovulation rate and consequently lambing rate in the Ghezel ewes.

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Table 1. The mean (±SEM) number of small, medium and large follicles in the four treatment groups on the day of estrus (day 1).

Parameter	Estrus (day 1)			
	Group 1	Group 2	Group 3	Group 4
Small follicles				
Right ovary	1.53±0.51	1.49±0.48	1.55±0.60	1.43±0.49
Left ovary	1.33±0.39	1.38±0.51	1.45±0.45	1.30±0.40
Total	2.96±0.64	2.83±0.57	3.05±0.92	2.70±0.79
Medium follicles				
Right ovary	1.57±0.41	1.51±0.45	1.53±0.44	1.48±0.55
Left ovary	1.43±0.35	1.37 ±0.42	1.42±0.38	1.35±0.46
Total	3.05±0.52	2.87±0.76	2.90±0.78	2.74±0.95
Large follicles				
Right ovary	0.68±0.39	0.71±0.40	0.69±0.43	0.51±0.55
Left ovary	0.89±0.43[a]	0.92±0.54[a]	0.91±0.46[a]	0.57±0.61[b]
Total	1.55±0.77[a]	1.67±0.89[a]	1.63±0.78[a]	1.11±0.90[b]

Different superscripts in the same row a significant difference (P < 0.05).

Table 2. Reproductive performance of Ghezelewes treated with various doses of PMSG following the CIDR Removal

Parameters	Group 1 350 IU (n=40)	Group 2 450 IU (n=40)	Group3 550 IU (n=40)	Group 4 (control) Saline (n=17)
Estrus response (%)	95.0 (38/40)	92.5 (37/40)	97.5 (39/40)	82.35 (14/17)
Pregnancy rate (%)	70.0 (28/40)	77.5 (31/40)	80.0 (32/40)	64.70 (11/17)
Lambing rate (%)	67.5 (27/40)	77.5 (31/40)	77.5 (31/40)	58.82 (10/17)
Litter size (%)	1.18 (32/27)	1.29 (40/31)	1.32 (41/31)	1.10 (11/10)
Gestation period(day)	154±0.24^{b}	150±0.26^{c}	149±0.18^{c}	157±0.30^{a}
Birth weight(kg)	4.7±0.11^{a}	4.2±0.11^{b}	4.1±0.10^{b}	4.9±0.12^{a}

^{a,b,c} :Means in the same row with different superscripts differ significantly (p< 0.05)

Figure 1. Ultrasound machine (Piemedical, Falco100; Holland, 8 MHz)

Figure 2. Ultrasound image of large sized follicles at estrus day on the left ovary

RESEARCH ARTICLE

Plasma Malondialdehyde, Adenosine Deaminase and Homocysteine Increase in Sertraline Affected Rat

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ABSTRACT

In the present study, we aimed to appraise whether malondialdehyde (MDA), homocysteine (Hcy) and activity of adenosine deaminase (ADA) are altered with sertraline administration in rat.Twenty four albino male Wistar rats were assigned into two groups and sertraline administered (20 mg/kg/day) to one of groups for 90 days in distilled water by stomach gavage and the other group not received any drug. Then, the plasma level of MDA was measured by the spectrophotometric method, ADA activity was identified with the electrochemiluminescence (ECL) technique and Hcy was measured using a RA1000in accordance with the ELISA method both in treatment and control groups.Significant increase ($p<0.01$) in MDA,Hcy and ADA activity ($p<0.01$) revealed in the treatment group compared with control ones. The results suggested that sertraline administration associated withincrease of those parameters may at least in part utilize in human medicine.

Key words: Sertraline, Biochemical profiles, rat.

INTRODUCTION

Reactive Oxygen Species (ROS), are continuously generated during metabolic processes. Oxidative stress is formed due to either ROS overproductionor shortage in antioxidant system and can stimulate some reactions leads to cellular

damage or cell death. Oxidative stress influences polyunsaturated fatty acids of cell membrane lipids and provokes lipid peroxidation which is used as determinant of oxidative stress and cellular injury indicator[1,3]. Malondialdehyde, as one of the lipid peroxidation by-products, is most abundant and reliable biomarker for assessing lipid peroxidation. Among neuropsychiatric diseases, the major depressive disorder (MDD)associated with oxidative stress[7].

Adenosine deaminase (ADA) has been considered as a substantial enzyme in the maturation and function of T lymphocytes. It is involved in the metabolism of purine nucleosides and plays an essential role in the immune, nervous and vascular systems[11].It has been considered as a major enzyme in the differentiation of T lymphocytes and its activity is higher in T cells than B cells. The plasma activity of ADA has been indicated to be elevated in inflammatory diseases with a cell mediated immune response. As an indicator of cellular immunity and T cell activation, it is related to ROS and NO^- generation by neutrophils. There is an association between activated T cell functions and lipid peroxidation in non-pregnant subjects that revealed interrelationship in both lipid peroxidation and T-cell activation[9]. In addition, an association has been reported between anxiety and purine metabolism [2].

Hcy is a sulfur-containing amino acid which is produced from the metabolism of methionine. In this metabolism, folate and vitamin B12 contribute the methylation of Hcy to methionine and their deficiencies are initial determinants for its elevation. High concentration of Hcygenerates oxidative stress, a risk factor for atherosclerosis, cell damage, toxic to vascular endothelial cells and neuronal cells but have also been involved with several psychiatric and neurodegenerative disorders including depression , schizophrenia and alzeimer disease[14].

To our knowledge, this is the first study evaluates the sertraline effectson MDA, ADA andHcy in rat.

MATERIAL AND METHODS

In this trial twenty four adult Albino male Wistar rats weighting 175±21g wererendered from animal house of Urmia University. Rats assigned into 2 groups in special cages and allowed to usewater, standard pellet ad libitum and 12:12 h light: dark at temperature 21-25°C with 39% humidity .Ethical Committee of the veterinary faculty of Islamic Azad University confirmed the experimental protocol of the study.

After 10 days acclimatizing, sertraline(Sigma Chemical Co. St. Louis, MO, USA) administered (20mg/kg/day)to one of groupsfor90 daysthrough stomach gavageand another group not received any drug. After 3 months, all rats were anesthetized(with sodium pentobarbital, 50 mg/kg, i.p) and blood sampling carried out via cardiac puncture, transferred to heparinized tubes and centrifuged 4000 RPMfor10 minutes at 4°C and someplasma thyroid function parameters (TSH, a-TPO, a-Tg and Tg) and ADAdetermined byelectrochemiluminescence technique (Roche Co. Elecsys 2010, Germany).

MDA level was detected by the Satoh method(spectrophotometer, model Cecil, Italy) and Hcy wasmeasured by ELISA technique (RA1000).

Data analysis was carried out with SAS Version 9.1 and Statistical methods accomplished in all analytes. Mean ± SD and determination of variation between the data results were expressed with Student's t test and significance level was set at ($p<0.01$).

RESULTS AND DISCUSSION

All of the altered parameters are denoted in Table1. The results indicated significant elevation of MDA, Hcy, TSH, aTg and ADA activity ($p<0.01$) in the treatment group in comparison with the healthy ones. In contrast, no significant changes revealed in the levels of aTpo and Tg in the treatment group ($p<0.01$).

Neurons and glial cells are vulnerable to oxidative stress than other cells and they consist of polyunsaturated fatty acids with partly low levels of antioxidants that is prone to oxyradicals damage. Thus, enhancement of free radicals (FRs) generation and/or decrement in their elimination in CNS cells may cause oxidative stress in neuronal cells[3]. Oxidative stress is characterizedby MDA elevation as a lipid peroxidation index. MDA is accompanied by different harmful effects on the cell membrane, such as increment in permeability and cross-linking with membrane ingredients[1]. Moreover, MDA possesses deleterious effects on serotonin receptors and may cause the destruction of neuron membrane phospholipids, membrane viscosity alterations and finally may impressserotonergic receptor functions [3]. The present study showed that sertraline administration causes MDA elevation than the control group. We could not find any information in the literatures about plasma MDA changes in SSRIs (e.g., sertraline) in rat. No changes in oxidative parameter (MDA) during 6 weeks of treatment with various antidepressants (venlafaxine, reboxetine + sertraline, sertraline or reboxetine) have been reported[15]. In contrast, Biliciet al.[5]demonstrated a decrease in the oxidative stress parameters in major depressive disorder patients during SSRI treatment.Furthermore, Kotan et al. [12]revealed decrease of MDA in long term antidepressant treatment in major depressive disorder. There is anassociation between ADA and FRs production through neutrophils function and FRs initiate lipid peroxidation. Therefore, MDA elevation may be associated with over activity of ADA. The other probable cause of MDA elevation is hyperhomocysteinemia. Hcy plays important role in ROS production. WhenHcy is liberated to plasma,it is promptly auto-oxidized and free radicals including superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are generated during sulfhydryl group oxidation. In addition, both O_2^- and H_2O_2promote lipid peroxidation and finallyHcy mediated free radicals overproduction along with lipid peroxidation generates inflammation[4].

The ADA activity was significantlyincreased in the present work. Herken et al.[10]reported elevation of ADA in major depressive patients during antidepressant therapy and concludedits activity may put in diagnostic enzymology upon in major depressive patients. In contrast,Elgun et al.[8]demonstrateda decrease of ADA activity level in major depression. Enhancement of ADA activity is related to T cells associateddiseases (cell-mediated immunity), as well as, it is known to play asa T cell activation indicator.On the other hand, it is incorporated with free radical production (NO^-, $O2^-$, H_2O_2 , OH^-) through neutrophils.Hence, a probable reason of high ADA activity may be due toover activity of cell mediated immunity.ADA irreversibly converts adenosine into inosine. Adenosine possesses strong anti-inflammatory effect which regulates the function of cells involved in the inflammatory reactions. It inhibits neutrophil-mediated injury to endothelial cells and modulates leukocyte–endothelial interactions. Accumulation of adenosine and deoxyadenosine cause to deleterious effect on cells, so their catabolism are very momentous for normal cells. Hence, the second probable reason of high ADA activity can be due to catabolic effect on adenosine degradation.In addition, Blardi et al. [6]reported that citalopram administration in depressed patients leads to adenosine elevation. On the other hand, sertraline administration may induce over production of adenosinein cellsand ADA inhibits harmful effects of adenosine through its catabolism.

Hcy involves in the pathophysiology of many neuropsychiatric disorders and neuron impairment andmay contribute tothe induction of apoptosis in neurons. Kruman et al. [13]revealeda direct effect of Hcy on hippocampal neurons of rat and activation of poly-ADP-Ribose polymerase (PARP) and NAD depletionwhich promotes oxidative stress, mitochondrial dysfunction and neuronal apoptosis. We could not find any information in the literatures concerning plasma Hcy alterations in SSRIs (e.g., sertraline) in rat. Folate and vitamin B_{12} associate with methionine-Hcy metabolism and involve in differentiation, development and function of the CNS. Recent study significantlyindicates high levels ofHcy in the treatment group compared to those of controls. Since a link betweenHcy and folate has been

determined, the significant rise of Hcy suggests failure of methylation of Hcy to methionine due to folate shortage(in the methyl folate form), as well as, direct effect of sertraline on methionine cycle is hypothesized on Hcy elevation.

In conclusion, the results in this study refer considerable increases of plasma MDA, ADA,Hcyin comparisonwithcontrolones and there is a probable relation between MDA with ADA activity and Hcy in rat.

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Table 1. Alterations in plasma thyroid indices, MDA, ADA and Hcy levels in the control and treatment groups.

Number	Parameter	Treatment group	Control group	P value
12	MDA(nmol/ml)	9.38±0.72†	4.01± 0.51	0.000
12	Hcy (U/L)	11.87 ± 2.23†	5.31 ± 0.43	0.000
12	ADA(U/L)	21.43 ± 2.65†	10.12 ± 0.67	0.000

Data are expressed as mean ± standard deviation, † Significantly different from the control group (P<0.01)

RESEARCH ARTICLE

Thyroid Function Indices Alter in Sertraline Affected Rat

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ABSTRACT

In the present study, we aimed to appraise whetherthyroid function indices such as, TSH, antithyroperoxidase (a-Tpo), antithyroglubolin (a-Tg), thyroglobulin (Tg) levels are altered with sertraline administration in rat.Twenty four albino male Wistar rats were assigned into two groups and sertraline administered (20 mg/kg/day) to one of groups for 90 days in distilled water by stomach gavage and the other group not received any drug. Then, the plasma level of thyroid indices were identified with the electrochemiluminescence technique both in treatment and control groups.Significant increase ($p<0.01$) in TSH and significant decrease ($p<0.01$) in a-Tg revealed in the treatment group compared with those of the controls. No significant changes observed in a-Tpo and Tg.The results suggested that sertraline administration associated with alterations of those parameters may at least in part utilize in human medicine.

Key words: Sertraline, Thyroid function indices, rat.

INTRODUCTION

The thyroid function is conducted with Hypothalamus-Pituitary-Thyroid axis (HPT) and drugs impress thyroid gland[7].Selective Serotonin Reuptake Inhibitors(SSRIs), a class of antidepressant drugs, increase extracellular levels of serotonin with inhibition of its reuptake into the synaptic cells[3].Sertraline (SSRI) belongs to naphthylamine derivatives, acts as antidepressant and antianxiety feature[8].More studies dealt to contradictory findings of tricyclic

antidepressant on thyroid function indices[4,10],whereas,in recent years, SSRIs are more pronounced than tricyclic antidepressants in daily psychiatric practice.As a part of this study, decreasing effect of sertraline in serum thyrotrophin (TSH) and an increase in serum thyroxine (T4) level has been reported[2].

Antithyroid antibodies (ATAs) involve in autoimmune thyroid dysfunction which impress microsomal antigen of follicular cell that is known as thyroid peroxidase (TPO) and high concentration ofthyroid peroxidase antibodies (TPOAb) is considered as a marker of autoimmune thyroid disease.Also, theassociation between the presence of TPO antibodies and autoimmune hypothyroidism development have been documented[15]. Among the various drugs, phenothiazines can persuade autoimmune thyroid dysfunction, as arecontributedinthe generation of antithyroglobulin (a-Tg) or antithyroperoxidase (a-Tpo) antibodies[13].

Since, the thyroid dysfunction is subsequentlyinducedby asymptomatic autoimmune thyroiditis, it remains still unclear to be characterized whether rats receiving sertraline maybe at risk ofATAselevation[9].

To our knowledge, although there isa paucity of studies related to sertraline effects on thyroid hormones but this is the first study evaluates the sertraline effectsonthyroid indices such as, TSH, a-Tg, a-Tpo and Tg in rat.

MATERIAL AND METHOD

In this trial twenty four adult Albino male Wistar rats weighting 175±21g wererendered from animal house of Urmia University. Rats assigned into 2 groups in special cages and allowed to usewater, standard pellet *ad libitum* and 12:12 h light: dark at temperature 21-25°C with 39% humidity .Ethical Committee of the veterinary faculty of Islamic Azad University confirmed the experimental protocol of the study.After 10 days acclimatizing, sertraline(Sigma Chemical Co. St. Louis, MO, USA) administered (20mg/kg/day)to one of groupsfor90 daysthrough stomach gavageand another group not received any drug. After 3 months, all rats were anesthetized(with sodium pentobarbital, 50 mg/kg, i.p) and blood sampling carried out via cardiac puncture, transferred to heparinized tubes and centrifuged 4000 RPMfor10 minutes at 4°C and someplasma thyroid function parameters (TSH, a-TPO, a-Tg and Tg)determined byelectrochemiluminescence technique (Roche Co. Elecsys 2010, Germany).Data analysis was carried out with SAS Version 9.1 and Statistical methods accomplished in all analytes. Mean ± SD and determination of variation between the data results were expressed with Student's t test and significance level was set at ($p<0.01$)

RESULTS AND DISCUSSION

All of the altered parameters are denoted in Table1. The results indicated significant elevation of TSH, aTg ($p<0.01$) in the treatment group in comparison with the healthy ones. In contrast, no significant changes revealed in the levels of aTpo and Tg in the treatment group ($p<0.01$).

TSH plays an important role in survey of major depression[1].The contradictory findings regarding TSH alterations have been reported in some literatures[5,12].Moreover,no significant correlation between antidepressant therapy with sertraline and thyroid releasing hormone-induced TSH wasshowed by Schule et al.[14]determined significant decrease of TSH in fluvoxamine treated patients. In the present study, TSH levelto be found high. The possible reason may be attributed to sertraline effects on HPT axis.

Antithyroid antibodies (ATAs) (a-Tg and a-Tpo) are known toplay as markers of auto-immune thyroid diseaseand thyroid dysfunction resulting in different degrees of hypothyroidism from subclinical to overt are attributed to ATAs[11]. There appears to be noreport on the levels of these indices to compare with the present findings in sertraline affected rat.some studies have exhibited ATAs in different disorders such as, positive a-Tg in patients with

premature menopause and the relation of autoimmune thyroiditis and minor hypothyroidism with SLE[6]. Moreover, autoimmune thyroiditis occurrence has been determined duringantipsychotic-induced hyperprolactinemia[9].Zelaya et al.[15] reported high prevalence of a-Tpo antibody in high–normal range TSH patients rather than low-normal range. Finally, phenothiazine derivated antidepressant drugs increases the production of ATAs (a-Tg and a-Tpo) and induces autoimmune thyroid dysfunction[13].

Present study indicated a significant rise in a-Tg compared to those of the control group and no significant changes in Tg and a-Tpo. Since an association between ATAs and thyroid disorders has been demonstrated, it is possible that sertraline administration causes thyroid dysfunctionwith unknown mechanisms in rat.

In conclusion,the results in this study refer considerable increases of plasma MDA, ADA,Hcy and alteration of thyroid indices compared to control ones and there is a probable relation between MDA with ADA activity and Hcy in rat.

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Table 1. Alterations in plasma thyroid indices, MDA, ADA and Hcy levels in the control and treatment groups.

Number	Parameter	Treatment group	Control group	P value
12	TSH(mIU/ml)	0 .365 ± 0 .056†	0 .074 ± 0 .11	0.001
12	aTg(IU/ml)	7.80 ± 0 .64†	4.21 ± 0 .23	0.000
12	aTpo(U/ml)	0.018 ± 0 .007	0.023 ± 0.012	0.411
12	Tg(ng/ml)	0.21 ± 0.13	0.11 ± 0 .05	0.051

Data are expressed as mean ± standard deviation, † Significantly different from the control group (P<0.01)

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RESEARCH ARTICLE

Identification of 2 Novel SNPs in Caprine DGAT1 Gene and its Association with Milk Traits in Indigenous Mahabadi Goats

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ABSTRACT

Characterization of milk production genes in different goat breeds is per-require and could substantially improve the efficiency of breeding programme. Therefore, the objective of present study was identification of novel polymorphism in exon 17 region of DGAT1 gene and its association with milk traits in 79 Mahabadi goat using PCR-SSCP tehnique and sequencing. In overall, four SSCP patterns were observed in gel with overall frequency 0.25, 0.24, 0.06 and 0.45, respectively. Subsequently after sequencing of each unique patternm 6 mutations was identified which 4 mutations of them were previously reported in other goats breed. However, two novel SNP identifed was located on 8290 position (-/C), which respectable frequencies of 0.308 and was 0.692 for present of C and absence respectively. Another novel mutation take placed in 8384 position (T→C) with frequencies of 0.054 and 0.946 for C and T alleles respectively. milk traits include milk production (g), milk fat and protein(%) and SCC (somatic cell count) were measured. The findings showed that DGAT1 gene had significant effect on milk production ($p<0.01$), fat percent ($P<0.05$) and protein percent ($P<0.05$), polymorphism of DGAT1 gene and its relationship with milk trait indicated that it can be useful in marker assisted selection programs.

Key words: DGAT1 - polymorphism - Sequencing - Mahabadi goat-milk trait

INTRODUCTION

Indigenous farm animal breeds are valuable gene pools for adaptive and economic traits, providing diversified genetic pool, which can help meeting future challenges. The importance of goats as a potential source of meat and milk has been realized particularly in small farming system in developing countries. (Latif et al. 1987). Dairy goat is considered the cow of the poor. The goat eats little, occupies a small area and produces enough milk for the average unitary family, whereas maintaining a cow at home cannot be afforded by the homeowner, hence, the growing popularity of goat as the poor person's cow. In developing countries, much of the milk produced by goats is for family consumption, but goat milk can also be further processed into a variety of marketable products (Aziz 2010).

The world total numbers of goats and sheep were 861.9 and 1078.2 million, respectively, i.e. there is about one goat to approximately 1.25 sheep in the world. The amount of goat milk produced in Iran was 25.3 (million), number of dairy goats and the average amount of milk produced by a dairy doe were 13.7(million), 29.9(Kg), respectively (FAOSTAT 2008). There are 25 million goats and 11 breeds in Iran that produce a variety of products for example Korki of South Khorasan and Korki of Raeini breeds for cashmere, Markhiz for mohair, Tail, Najdi and Black Lori breeds for milk and meat products and indigenous Mahabadi goats for milk product.

Barllett (2007) studied the genetic improvement of dairy goats and sheep and reports that goats and sheep breeding in the past decade has been the primary only based on quantitative genetics information. At the same time, a new window called the selection or genetic marker-assisted (MAS-GAS) is open in Molecular Genetics. It seems that in the near future breeding programs in dairy goats and sheep depended on the use of technology and information molecular genetics (Barillet 2007). Disadvantages breeding goat is lack pedigree and appropriate record system. The purpose of breeding goat is mainly production of milk and meat in Iran.

New molecular techniques focused on genome analysis open new possibilities for complex evaluation of economically important traits in farm animals (Madeja et al. 2004). Diacylglycerol acyltransferase1 (DGAT1) gene suggested as a functional candidate gene for milk production traits (Smith et al. 2000; Winter et al., 2002) encodes an enzyme which plays a major role in the synthesis of triglycerides. Nucleotid sequence analysis of this gene has revealed that the bovine DGAT1 gene includes 17 exons of variable size encoding a 489 amino acid protein(Grisart et al. 2002). A base substitution (K232A) in the DGAT1 (the acyl-CoA: diacylglycerol acyltransferase1) gene at position 10,433 and 10,434 in exon number 8 lead to QTL (quantitative trait loci) variation (Grisart et al. 2002). While Lysine variant (K allele) of DGAT1 gene was associated with high fat yield, Alanine variant (A allele) of DGAT1 gene was associated with high milk yield.

Characterization of milk production genes in different goat breeds is per-require and could substantially improve the efficiency of breeding programme. The character of Mahabadi Goat is white coat with black spots, 2 kg at birth weight and 50 kg at mature weight for female and 55-60kg for male and above 50 percent is twining. The average milk production is 1.6 kg for this breed. This breed is mainly distributed in the region of Kurdistan.

The main objective of present study was identification of novel SNP of exon 17 of DGAT1gene and its association with milk traits in Mahabadi goats using PCR-SSCP and PCR- sequencing.

MATERIALS AND METHODS

Animals

In overall, blood sample collected of 150 Mahabadi goats belong to the Department of Animal Science the University of Tehran (Karaj). Sampling was done randomly, that were included male and female goats and kids, and was recorded of 79 Mahabadi goats for milk production during once in two week s for2 years(3 months per year) .The structure of pedigress was included age of dam, weight of dam and moon, season and year of parturition and record's of trait were measured. The measured traits such as milk production, fat and protein percent. Date of recording for milk production was from 2009- 2011 and for fat, protein percent and SCC was from 2009-2010. Number of records used for milk production was 864 and for fat and protein percent and SCC were 244.

Molecular study

Blood samples were collected from jugular vein and was transferred into 5mL tubes containing EDTA to prevent coagulation and then stored at 4 °C until DNA extraction. DNAs were extracted faccording to conventioal Salting out) method (Iranpur and Esmailizadeh., 2010. The existence and concentration of DNA was checked by agarose gel electrophoresis (1% agarose gel and spectrophotometer. A 309-bp fragment of sheep DGAT1 gene was amplified using PCR procedure applied by Zhou in 2008 (Xu et al. 2008). Primers given below were used for amplification of 309-bp fragment of exon 17 of DGAT1 gene**:**

Forward: 5´-GCA TGT TCC GCC CTC TGG-3´

Reverse: 5´-GGA GTC CAA CAC CCC TGA-3´

The PCR was performed with 25 µL reaction mixture containing 10 pmol of forward primer and the same amount of reverse primer, 25 mM/µL $MgCl_2$, 10 mM /µL dNTPs (dATP, dTTP, dCTP and dGTP), 10x Buffer, 5unit/µL of Taq DNA polymerase, and 50 ng caprine genomic DNA. Materials and equipment required for PCR were obtained from Cinnagen company.A total of 35 cycles were adapted for denaturation at 94°C/30 s, annealing at 57°C/30 s and polymerization at 72°C/30 s using in a T-Personal thermo-cycler. Bioer PCR equipment used in this research was purchased from Techne Company (Britain).

PCR-SSCP

SSCP method was used to scan mutations within the amplified regions. Aliquots of 6 µL PCR products were mixed with 12 µL denaturing solution (95% formamide deionized, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 95°C and chilled in ice immediately. Denatured DNA was subjected to 12% PAGE (polyacrylamide gel electrophoresis) in 1X TBE buffer and constant voltage (300 V) for 21 h at a constant temperature of 4°C, and then gels were stained with silver nitrate, during the three stages of fixer, stainer and developer, and staining continued untill bands appeared (Bassam et al.1991; Jin et al. 2010).

After SSCP, among samples with different banding pattern, three samples of each pattern for sequencing were sent to Bio Neer Company. The samples by Bio Edit software were sequenced.After sequencing, it was found that the second and fourth patterns have the same sequence and were in a group.

Statistical analysis

The genotypic and allelic frequencies, Shannon's Information Index, Hardy-Weinberg equilibrium were calculated by software GenAlex (Version 6.41). BioEdit software was used for sequence analysis, identification novel SNPs and Compare sequences of different species.The statistical software SAS (Version 9.1) was used to analyze variance milk traits in Mahabadi goats. Normal distribution of residuals for each trait in the population was analyzed using proc univariate of SAS 9.1 statistical program. The adjusted Linear Model with fixed effects was used to deal with the relationships between genotypes and milk traits of 864 records. Linear Model:

$$Y_{ijklm} = \mu + A_i + G_j + Y_k + M_l + b(W_{ijklm} - \overline{W}) + e_{ijklm}$$

Where:

y_{ijkm} = the trait measured on each of the ijklmth animal,
μ =the overall mean,
A_i =the ith age of the animal,
G_j =the type of the jth genotype,
Y_k = the kth year of kidding,
M_l= the lth Month of the recording
W_{ijklm}= mth animal of the weight at parturition
$\overline{W}$ =The mean weight of the animals at parturition
e_{ijklm}= the random effect of residual.

RESULTS

Identified SSCP pattern showed in overall four patterns band with frequency patterns 0.25, 0.24, 0.06 and 0.45, respectively which indicated the high polymorphism in this gene. Fragment of 309 bp DGAT1 gene was compared with sequence GenBank accession no. EU178818.1 introduced in NCBI. These sequences were Alignment and were compared with sequences available for Capra hircus, Ovis aries and Bos tauros sequences (Figure1).

Genotypic and allelic frequencies were presented in Table 1. The frequencies of T allele (Locus2) and genotype 1 were dominant in Mahabadi goat's population. The genotype distributions were not in agreement with Hardy–Weinberg equilibrium. sequencing results were showed in overall 6 mutations which out of 4 mutations were previously reported in other breed of goats.

However, two novel identifed mutations was located on 8290 position (-/C), which respectable frequencies of 0.308 and was 0.692 for present of C and absence respectively. Another novel mutation take placed in 8384 position (T→C) with frequencies of 0.054 and 0.946 for C and T alleles respectively. Correlation coefficients for milk production traits are shown in Table3. The results showed that correlation milk production with the percentage of milk fat, protein and somatic cell count was negative.

This study showed that dam age at the birth of kid had the significant effect on milk yield (p<0/01) and milk protein percentage (p<0/05). Year of parturition had significant effects on milk production traits (p<0/01). Dam weight had significant effect on milk yield (p<0/01) and milk fat percentage (p<0.05). Month of the recording had significant effect on milk production traits (p<0/01) and somatic cell count (p<0/05). The least squares mean comparison was observed

there are significant differences between genotype groups in all parameters except the number of somatic cell (Table 2**)**.

Genotype 1 had the most effect on milk production and genotype 2 had the most effect on fat percentage. The result of this research showed that DGAT1 gene had significant effect on milk production ($p<0.01$), fat percent ($P<0.05$) and protein percent ($P<0.05$).

DISCUSSION

DGAT1 plays a fundamental role in triacylglycerol synthesis**.** Identified SSCP pattern showed a in overall four patterns band with frequency patterns 0.25, 0.24, 0.06 and 0.45, respectively which indicated the high polymorphism in this gene. Kaupe et al (2004) reported DGAT1gene polymorphism in Holstein. Different cattle breeds show highly variable frequencies of the mutation (Kaupe et al. 2004). In this study two novel identifed mutations. The least squares mean comparison was observed there are significant differences between genotype groups in all parameters except the number of somatic cell. Spelman et al. (2002) were observed significant difference between genotype groups in DGAT1-CfrI locus. Correlation between the DGAT1 K allele and milk fat content was found by Kepenek et al. (2007). The K232A genotypes were significantly associated with milk fat percentage (KK, KA>AA [$P\leq0.005$, $P\leq0.05$]) and milk yield (KK, KA>AA [$P\leq0.05$, $P\leq0.005$]). The K allele was also favourable for SCC levels (Ivan Manga and Honza Ríha, 2011). The effect of DGAT1 gene on FAT2x (milk fat yield adjusted for two milkings per day, kg), FATP2X (fat content of milk adjusted for two milkings per day, %), EBVFP (estimated breeding value for fat content of milk, %) and PROPER305 (milk protein content adjusted for 305 days, %) occurred highly significant ($P<0.001$) **(**Kharrati Koopaei et al. 2012).Weller et al (2003) reported significant effects of DGAT1 locus on milk production traits in Holstein bulls. Grisart et al (2002) was found A two-nucleotide substitution in exon VIII of unprotected DGAT1 gene in the 10433 and 10434 positions, which was Causing an amino acid substitution at position 232 DGAT1 in bovine and had a large effect on milk fat. A Variety of lysine (K) DGAT1 was associated with high fat, whereas a variety of alanine (A) was associated with increased protein and milk production. Polymorphism of DGAT1 gene and its relationship with milk trait indicated that it can be useful in marker assisted selection programs. The results of several studies on the different locus of DGAT1 genes were present in Table 3.

CONCLUSION

According to the DGAT1 gene had significant effect on milk production ($p<0.01$), fat percent ($P<0.05$) and protein percent ($P<0.05$), Genotype effects on milk production as desirable genotypes used in breeding programs and generally polymorphism of DGAT1 gene and its relationship with milk trait indicated that it can be useful in marker assisted selection programs.

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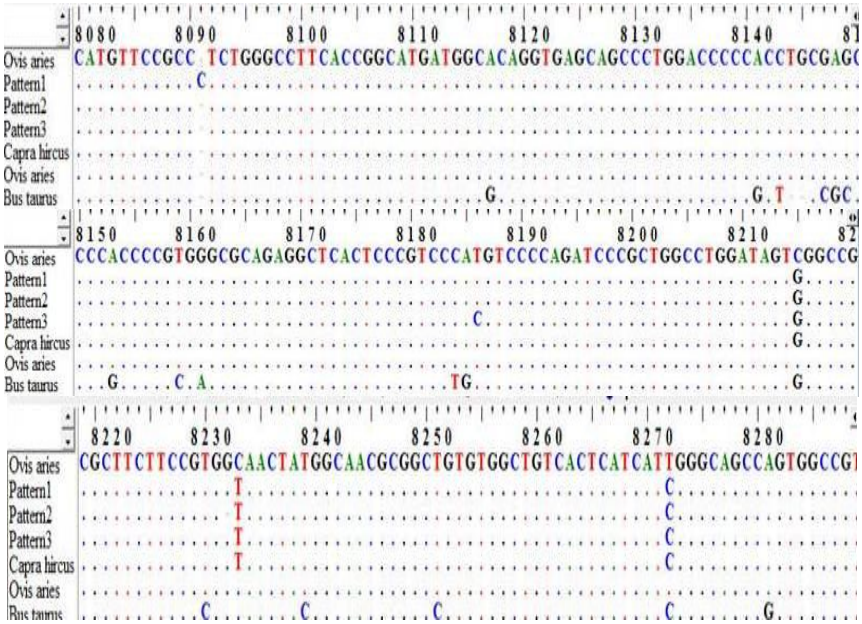

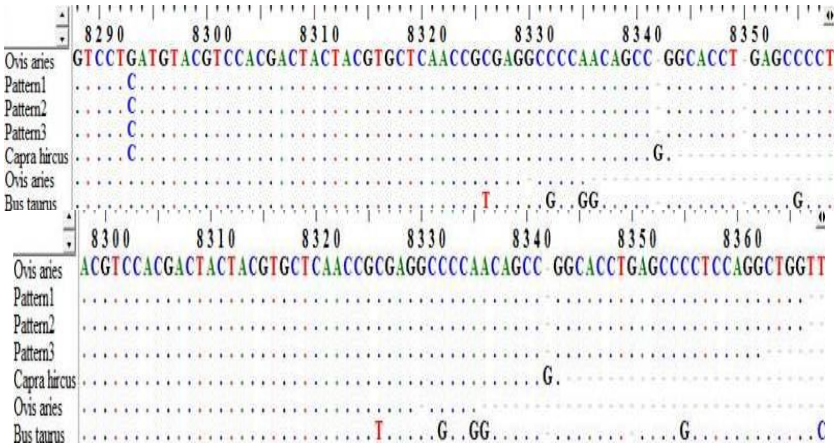

Figure 1- Sequence corresponding to NCBI, sequences in this study (goat Mahabadi sequence), Capra hircus, Ovis aries and Bos tauros sequences.

Table 1- Allele & genotype frequencies of DGAT1 gene in Mahabadi goats population.

Allel frequency	Locus1		Locus2	
	-	C	T	C
	0/692	0/308	0/964	0/054

Genotype	Nucleotide NCBI 8290	Nucleotide NCBI 8290	Genotype frequency
Genotype1	TT	- -	69
Genotype2	CC	- -	25
Genotype3	TT	CC	6

Table 2- Least Square Mean Comparison (±SE) of the DGAT1 gene effect on milk trait traits in Mahabadi goats population.

trait	Genotype1	Genotype2	Genotype3
Milk production(gr) **	(27.11± 0.92)^{a}	(28.54± 1.09) ^{ab}	(33.03± 1.26) ^{b}
% fat*	(0.51±0.21)^{b}	(1.05±0.28)^{a}	(0.69± 0.29)^{ab}
% protein *	(0.485 ±0.002)^{b}	(0.489 ±0.003)^{ab}	(0.491±0.003)^{a}
SCC ns	4.02±0.24	4.07±0.32	4.17±0.34

Ns, **,*, non-significant, significant at 1% and 5% level respectively.

Table 3- Summary of the results of different research on DGAT1 gene

Author and year	Region	Breed	Country	Molcular technique	SNP	Allele frquency	Association	Favorable allele
Patel *et al*., 2012	Intron 1	Bubalus bubalis	India	ABI-PRISM automated DNA sequencer	T→C	T-(0.08) C-(0.92)	There was no association of the SNPs genotyped was observed with MY(milk yield) and MFP(milk fat percentage)	-
Strzałkowska *et al*., 2005	Exon 8	Black and White (Friesian) cows	Poland	RFLP-PCR	AA →GC	AA-(0.40) GC-(0.60)	No significant differences were found between genotypes in the weight of milk components produced daily and on the daily milk yield. In particular the *AA* allele (coding for lysine) appeared favourable for fat and protein content of milk	AA
Xu *et al*., 2008	Exon 17	lambs of three Chinese breeds	China	RFLP-PCR	GCT→GCC	T-(0.62, 0.59, 0.74) For three breeds	The T allele had a positive effect on tenderness, IMF content and marbling score in sheep meat	T
Grysart *et al*.,2004	Exon 8	Black and white Holstein Friesian	Belgium	OLA	K→A	-	Genotypes were associated with increased protein, fat and milk production	A
Scata (2009)	5′ UTR Intron2	three Italian sheep breeds	Italy	(DHPLC) Transgenomic WAVE _system	C>A C>T	(0.05 - 0.02) 0.19 (0.25 - 0.32 - 0.41)	-This SNP(C>A) showed a significant negative association with milk fat content in the Sarda sheep. This SNP(C>T)showed a negative association with milk fat content.	C>A
(Kharrati Koopaei *et al*.20	Exon 8	Iranian Holstein cattle	Iran	RFLP-PCR	K→A	K- 0.37 A- 0.63	The effect of *DGAT1* gene on FAT2x, FATP2X EBVFP and PROPER305 occurred highly significant.	A

12).								
Kepenek *et al*.,2007	Exon 8	Turkish native cattle breeds	Turkish	RFLP-PCR	K→A	Kallele higher than the A allel in all breeds	Genotypes were significantly associated with milk fat percentage, and milk yield	A
Angiolillo *et al* 2006	Intron 16	Murciano Granadina-Malaguena-Saanen-Girgentana Goat breeds	Spain	RFLP-PCR	T→C	T allele higher than the C allel in all breeds	Not expected to have any functional effect, might be useful as a genetic marker in association studies to detect additional DGAT1 polymorphisms which might influence fat milk content and other traits of economic interest.	C
Parek *et al*.,2005	Exon 8	Polish Black and White cattle	Poland	RFLP-PCR	K→A	AIsires(0.60(k) 0.40(A)). Youngbulls(0.68 - 0.32)	The Kruskal-Wallis test revealed a highly significant effect of *DGAT1 K232A* in cows with extremely low fat content and a significant effect in cows with extremely high protein content of milk	A
Scotti *et al*., 2010	Exon 8	Italian cattle breeds	Italy	RFLP-PCR	K→A	K(0.25)-A(0.75).	-	-

RESEARCH ARTICLE

Identification of One Novel SNP in Third Exon of Caprine Leptin Gene in Indigenous Mahabadi Goats Using PCR-SSCP and Sequencing

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ABSTRACT

Leptin, a 16-kDa protein secreted from white adipocytes, has been implicated in the regulation of feed intake, energy expenditure, and whole-body energy balance, reproductive, immunity, growth and obesity gene product (obese) coded by a gene called ob gene. The present study has been designed to determine polymorphism in third exon of leptin gene in Mahabadi goat using PCR-SSCP. Blood sample were collected from 150 Indigenous Mahabadi goat belong to the Department of Animal Science the University of Tehran (Karaj). DNA was extracted using Salting out method. DNA quality and quantitative was determined with spectrophotometer and 1% agarose gel. A fragment of third Exon of leptin gene was amplificated using the polymerase chain reaction. SSCP (single strand conformation polymorphism) technique was used to study polymorphism of this fragment of DNA. In this regards samples were run in 12% polyacrylamide gel electrophoresis for 21h with 300Voltage. Gels stained with silver nitrate, in three stages of fixer, stainer and developer. Developing pattern show the same pattern in all animals and were monomorph. The results of this research were showed 6 mutations for this locus that the Indigenous mahabadi goats breed for these mutations are monomorph and 5 mutations ordinary was reported in other breeds of goats. One novel SNP identified in this research was substitution A→G at 3145 nucleotide position.

Key words: Leptin - polymorphism - PCR-SSCP - Mahabadi goat.

INTRODUCTION

Goats disseminated all over the world because their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained (Aziz 2010). They proved useful to man throughout the ages due to their productivity, small size, and non-competiveness with him for food. Goats are mainly kept: to produce milk, meat or fiber (Mohair and Cashmere).

The world total numbers of goats was 861.9 million. There is about one goat to approximately 1.25 sheep in the world (FAOSTAT 2008). There are 25 million goats and 11 breeds in Iran that produce a variety of products for example Korki of South Khorasan and Korki of Raeini breeds for cashmere, Markhiz for mohair, Tail, Najdi and Black Lori breeds for milk and meat products and indigenous Mahabadi goats for milk product.

A global overlook of the goat industry indicates that few well organized selection programs have been developed, although goats have the biggest increase in number among the livestock species during the last 20 years (Dubeuf and Boyazoglu 2009). In many parts of the world where the geophysical properties of the terrain are not suitable for other livestock species, goats seem to be the best choice. The role of goats in supplying food to humans has been well stated by many researchers (Devendra 1985). The breeding program should also be flexible and allow the incorporation of new selection criteria.

Leptin, a 16-kDa protein secreted from white adipocytes, has been implicated in the regulation of food intake, energy expenditure, and whole-body energy balance in rodents and humans (Houseknecht et al. 1998). Leptin gene as a QTL has been considered and are affected the economic traits such as milk and meat production and reproduction. Liendersoon et al (1998) has been reported a QTL affecting milk production traits on chromosome four in the area which leptin gene and amylase -1 have been located (Liendersoon et al. 1998).

The character of Mahabadi Goat is white coat with black spots, 2 kg at birth weight and 50 kg at mature weight for female and 55-60 kg for male and above 50 percent is twining. The average milk production is 1.6 kg for this breed. This breed is mainly distributed in the region of Kurdistan.

The main objective of present study was identification of novel SNP of exon 3 of leptin Gene in Mahabadi goats using PCR-SSCP and PCR- sequencing.

MATERIALS AND METHODS

Animals

In overall, blood sample collected of 150 Mahabadi goats belong to the Department of Animal Science the University of Tehran (Karaj). Sampling was done randomly, that were included male and female goats and kids.

Molecular study

Blood samples were collected from jugular vein and was transferred into 5mL tubes containing EDTA to prevent coagulation and then stored at 4 °C until DNA extraction. DNAs were extracted according to conventional Salting out) method (Iranpur and Esmailizadeh, 2010. The existence and concentration of DNA was checked by agarose gel electrophoresis (1% agarose gel and spectrophotometer. A 471-bp fragment of sheep LEPTIN gene was amplified

using PCR procedure applied by Zhou in 2009 (Zhou et al.,2009). Primers given below were used for amplification of 309-bp fragment of exon 3 of LEPTIN gene**:**

Forward: 5'- AGGAAGCACCTCTACGCTC -3'

Reverse: 5'- CTTCAAGGCTTCAGCACC -3'

The PCR was performed with 25 µL reaction mixture containing 10 pmol of forward primer and the same amount of reverse primer, 25 mM/µL $MgCl_2$, 10 mM /µL dNTPs (dATP, dTTP, dCTP and dGTP), 10x Buffer, 5unit/µL of Taq DNA polymerase, and 50 ng caprine genomic DNA. Materials and equipment required for PCR were obtained from Cinnagen Company. A total of 35 cycles were adapted for denaturation at 94°C/40 s, annealing at 62.5°C/30 s and polymerization at 72°C/30 s using in a T-Personal thermo-cycler. Bioer PCR equipment used in this research was purchased from Techne Company (Britain).

PCR-SSCP

SSCP method was used to scan mutations within the amplified regions. Aliquots of 6 µL PCR products were mixed with 12 µL denaturing solution (95% formamide deionized, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 95°C and chilled in ice immediately. Denatured DNA was subjected to 12% PAGE (polyacrylamide gel electrophoresis) in 1X TBE buffer and constant voltage (300 V) for 21 h at a constant temperature of 4°C, and then gels were stained with silver nitrate, during the three stages of fixer, stainer and developer, and staining continued until bands appeared (Bassam et al.1991; Jin et al. 2010).

After SSCP, Developing pattern show the same pattern in all animals and were monomorph. Among samples with same banding pattern, three samples for sequencing were sent to Bio Neer Company. The samples by Bio Edit software were sequenced. After sequencing, it was found that the patterns have the same sequence and there were in a group.

RESULTS

Identified SSCP pattern showed in overall same band patterns (Figure 1) which indicated the monomorph in this gene in population of indigenous Mahabadi goat. The results of this research were showed the Mahabadi goat breed for these mutations are monomorph.

Fragment of 471 bp leptin gene was compared with sequence GenBank accession no. HE605296.1 introduced in NCBI. These sequences were Alignment and were compared with sequences available for Capra hircus, Ovis aries and Bos tauros sequences (Figure1).

Results were showed in overall 6 mutations which out of 6 mutations were previously reported in other breed of goats, except, one novel identified mutation was located on 3145 position (A/G). This mutation has been reported in cattle and sheep. SNPes identified in this study that compared to nucleotide sequences HE605296.1 in the NCBI (table 1).

The amino acid sequence of the 471 bp fragment of exon 3

Amino acid sequence of a fragment of 471 bp compared before entering and after entering SNPs by the software Bio Edit. It was observed that a change in an amino acid resulting from substitution A→G at nucleotide position 3201

occurred and the amino acid Asparagine is converted to serine. In a study on sheep, four SNP was identified in exon 3 as the three SNP was associated with amino acid changes (Zhou et al. 2009).

GSTSTLEGKAELGSSEELPSLPLSSCCPLPPAENDQSVSSKQRVTGLDFIPGLHPLLSLSKMDQTLAIYQQILASLPSRN VIQISNDLENLRDLLHLLAASKSCPLPQVRALESLESLGVVLEASLYSTEVVALSRLQGSLQDMLRQLDLSPGC END SLEG

DISCUSSION

Leptin gene as a QTL has been considered and are affected the economic traits such as milk (Silva et al. 2002; Madeja et al. 2004) and meat production (Buchanan et al. 2002) and reproduction (Liefers et al. 2003). Identified SSCP pattern showed in overall same band patterns in all 150 goats and they were monomorph. In Hashemi et al (2011) study a 471 bp LEP exon 3 segment was amplified by standard PCR. PCR products were subjected to SSCP denaturation and polyacrylamide gel electrophoresis. Five SSCP patterns, representing five different genotypes, were identified (Hashemi et al. 2011). In a study by Dubey et al (2008) to identify the genetic variations of the entire leptin gene in Sahiwal cattle using a non-radioactive PCR-SSCP technique, no SSCP band patterns were detected in the LEP6, LPT9 and LEP12 regions under various electrophoretic conditions. However, analysis of the LEP1-LEP5, LEP8, LEP10, LEP11 and LEP13 fragments did reveal the polymorphisms analysis of the LEP1-LEP5, LEP8, LEP10, LEP11 and LEP13 fragments did reveal the polymorphisms (Dubey et al. 2008). In the present study results were showed in overall 6 mutations which out of 6 mutations were previously reported in other breed of goats (Sharma et al. 2012) except, one novel identified mutation was located on 3145 position (A/G), this mutation has been reported in Bos Taurus (Di Gregorio et al. 2011) and in ovis aries (Zhou et al. 2009). In Zhou et al (2009) study, variation in the exon 3 coding sequence of the ovine LEP gene was investigated by (PCR–SSCP) analysis and DNA sequencing. Five novel SSCP patterns, representing five different sequences, were identified under a combination of two different electrophoresis conditions. Four single nucleotide polymorphisms (SNPs) were detected, and three of these resulted in amino acid changes. In a study by Singh et al (2009) to analyze polymorphism in exon 2 and intron 2 region of leptin gene in Indian goats, Five major haplotypes were observed in exon 2 region and six major haplotypes observed in intron 2 region in both breeds(Singh et al. 2009). In azizi et al (2012) study on polymorphism of two loci of Ovine Leptin gene in Lori-Bakhtiari, Zel and Atabay-Zel crossbreds sheep, were observed 7 band patterns in exon 3(azizi et al. 2012). The fragment of exon 3 of the Leptin gene (275 bp) was characterized successfully and amplified for Kermani sheep by Shojaei et al (2010). In this study 10 conformational patterns were observed. Two single-nucleotide polymorphisms (SNPs) were genotyped, including the A59V in exon 3 and Sau3AI in intron 2. Statistical analysis revealed that the A59V polymorphism significantly affected the milk, protein, and fat yield (Kulig et al. 2009). In Matteis et al (2012) study on Association analyses of single nucleotide polymorphisms in the leptin and leptin receptor genes on milk and morphological traits in Holstein cows one novel SNP in the leptin gene were detected (Matteis et al. 2012).

CONCLUSION

Considering the high polymorphism this locus in cattle and sheep and role of this candidate gene in controlling food intake and fat storage and etc. it is recommended that further research be conducted with other breeds of goats and made comparisons between breed and analyzed differences.

ACKNOWLEADGMENT

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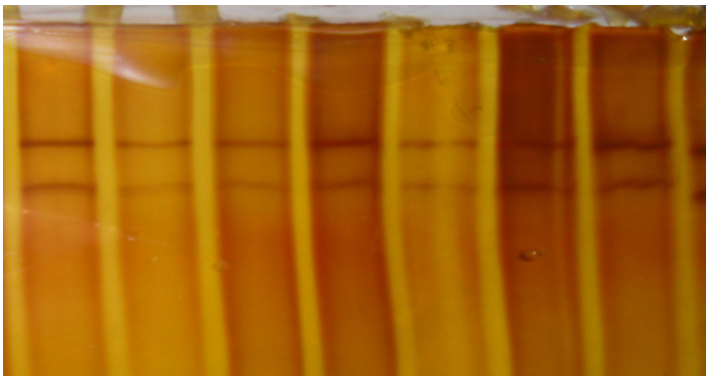

Figure1Band patterns of exon 3 of the leptin gene in Indigenous Mahabadi Goats.

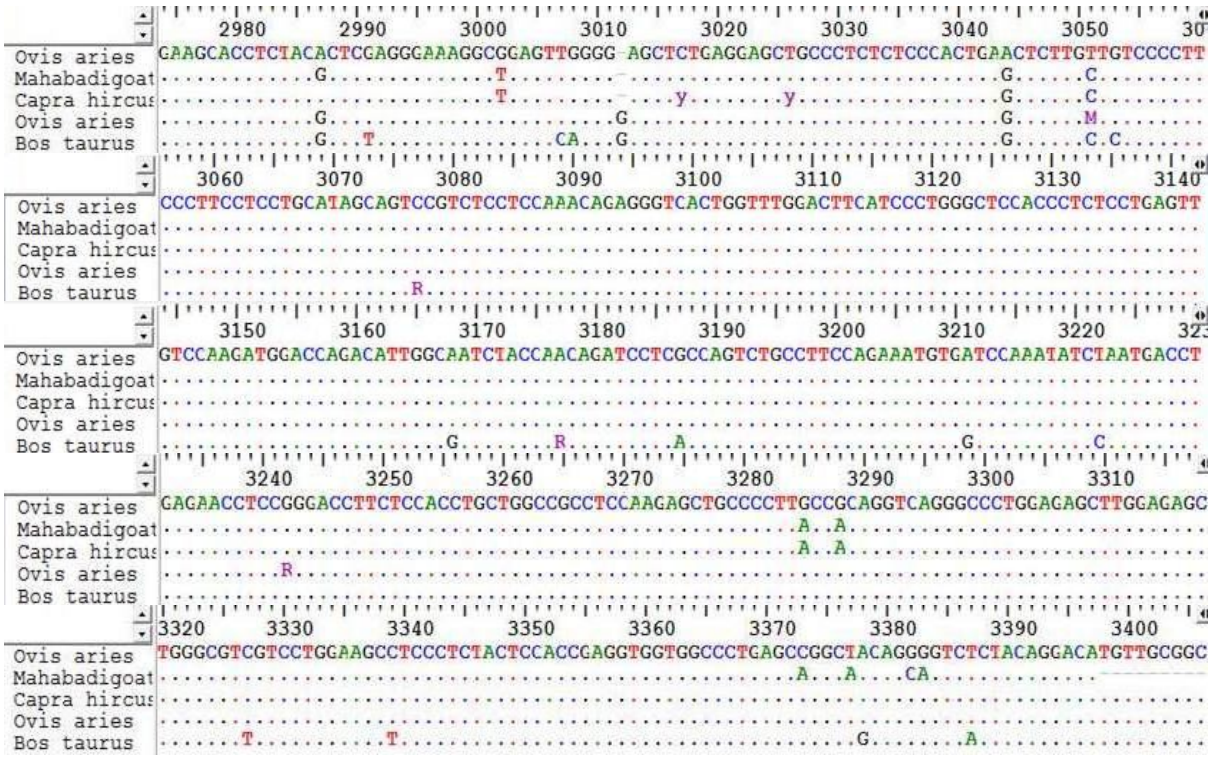

Figure 2- Sequence corresponding to NCBI, sequences in this study (goat Mahabadi sequence), Capra hircus, Ovis aries and Bos tauros sequences.

Table 1- comparison SNPes identified in present study and sequences HE605296.1 in the NCBI

The present study	Genbank: HE605296.1 Gregorio(2011)	Nucleotid position
G	A	3145
T	G	3160
G	A	3201
C	T	3208
A	G	3442
A	G	3445

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RESEARCH ARTICLE

Use of a Model for Determining the Probability of Working Days Suitable for Tillage

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ABSTRACT

The most important factor in order to do the work in specified time is estimating the number of workdays. Number of workdays in each region varies according to limiting factor or inhibiting, according to these factors must be determined in each area workdays.the limiting factors for tillage, including soil moisture, precipitation, there is residue on the soil surface, temperature and other competitive agricultural operations and labor is the source of power. Soil moisture was cited as the most important factor can be the limiting factor for tillage operations.

In this research, the validation of the model was designed to determine the probability of workdays tillage were investigated. For the simulation model inputs should be measured in accordance with the conditions which able to compare the model results with measured data. For this purpose, three field tests were conducted three cities in the province of Qazvin in Iran (Qazvin, Abyek and Bouin Zahra). the results of simulation models for estimating soil moisture, the soil moisture release curve and unsaturated hydraulic conductivity were compared with measured data.The results showed that the model has good accuracy. The input sensitivity of the initial moisture content of the soil, showed that the initial moisture content is important in the first few days and approached the input and output level or increase soil moisture, this time is reduced . In determining the probable workdays , where only the upper limit of workability as tillage limiting factor be considered, a relatively large probability obtained workday**.**

Key words: tillage, probable workday, workability, model, evaporation

INTRODUCTION

The most important factor in order to do the work in specified time is estimating the number of workdays. Number of workdays in each region varies according to factors limiting or inhibiting, according to these factors must be determined in each area workdays. Natural conditions, along with other factors which limits the programmer control over them. To estimate the number of workdays is likely to be sources of information such as weather data and expert opinion is required. For agricultural operations in the region, the scope of appropriate time to perform the operations if the operation of the limiting factor is not the proper time limit, will be a drop in yield. The advanced agricultural systems in each region considering to the weather conditions and the type of products which are grown, before starting to perform the operation, workdays for each operations should be predicted. Therefore, farmers according to the appropriate workdays perform in that area, the number of machines required to be calculated. In the advanced agricultural and science the need for proper crop calendar with detailed weather information for the Calendar suitable machine is inevitable. Required time to operate a farm machine depends on machine capacity and available number of workdays.

For doing of each operation, tillage, planting, plant protection and harvesting of each region according to the kind of product, there is an appropriate time. If due to the lack of agricultural calendar and machinery and lack of attention to the reliability of the machines, the operation is not performed in the range of proper time, causing the reduction in the value of product. The yield reduction caused the loss of value of production (in fact, some loss of profits),Those effects arenot realized and perhaps in many cases, Farmers did not notice to these losses even the amount of these losses of reduction in yield due to timeliness, Into account the lack of fertile soil, pests and diseases, inadequate distribution of rainfall and other factors effect on timeliness in addition to the quantity which has many effects in the quality, will impact on the price and marketability of products .

the limiting factors for tillage, including soil moisture, precipitation, presence of residue on the soil surface, temperature and other competitive agricultural operations and labor are the source of power. the most important factor which cited above is soil moisture for tillage operation. If tillage is done in an high humidity land, operations increased compaction and damage to the soil structure. If tillage is done in a very dry land, in addition to forming a large clods, due to the high resistance of the soil, the energy needed to perform increases.

Soil moisture content and the weather are the two major factors which determine the amount of time available throughout the year for field operations. In a poor season, little time may be available for performing one or several field operations under acceptable conditions. A favorable weather pattern and a firm soil, on the other hand, provide abundant time in which the field work can be completed without working excessively long hours, or working in unsatisfactory conditions. The weather interacts both with the soil to vary soil workability for tillage operations, whilst the influence of the weather on soil trafficability affects all operations to a greater or lesser extent .The workability and trafficability of the soil is dependent on the soil moisture content which can be evaluated from soil and weather variables (Witney 1988).

Rainfall alone is of little help in determining the moisture content of the soil because drainage of water through the soil varies markedly from site to site. Both Evaporation of moisture from the soil and transpiration of water vapour by the crop are also affected by the ambient temperature, with warmer intervals favouring a more rapid moisture loss. In addition, the existing moisture status of the soil governs the rate of drainage and the rate of evapotranspiration. As the soil dries out, the removal of additional water becomes progressively more difficult. Not accounting for this fact in the past has led to excessively high estimates of soil workdays, Lastly, during periods of high intensity or prolonged rainfall, the percolation of water through the soil is too slow to meet the deluge imposed upon it and the excess flows over the soil surface to the nearest waterway as runoff (Witney 1988).

These factors are combined to form the soil moisture balance equation (1) for the top 300 mm of the plough layer (Witney 1988): Soil moisture content, mm = Soil moisture content on the previous day, mm + Precipitation, mm - Runoff, mm - Drainage, mm - Evapotranspiration, mm, (1)

Soil workability is directiy related to soil moisture content. The soil moisture model can be used to predict the probability of occurrence of workdays when the soil moisture content is at or below a specific value or soil workability criterion over a period of years. As soil workability varies from soil to soil, machine to machine and farm manager to farm manager, the adoption of a unique soil moisture value to differentiate between soil workability and nonworkability is unrealistic. A procedure has been adopted, therefore, to enable the number of soil workdays to be calculated at different levels of soil moisture content or workability criteria. Usually suitable soil moisture, the lower limit of the plastic and or little less than the limit mentioned (Witney 1988).

The soil workability criterion is the soil moisture content at or below which the soil is workable.
The plastic limit or lower limit of the plastic state is the soil moisture content at which the soil begins to crumble when rolled out into thine threads

The similar concepts of soil workability and trafficability are not precisely defined in terms of the physical mechanisms controlling them. However, they are useful for assessing the opportunities for farm machinery to access land and consequently the timeliness of tillage operations. Trafficability is considered to represent the capacity of a soil to support and withstand traffic and provided that crop yields are not adversely affected, a soil is trafficable when soil structural damage is absent or negligible.

Workability describes the condition of a soil when tillage operations (ploughing and seed-bed preparation) can be performed. So, if seed-bed preparation is the required operation, a soil is considered workable when conditions are suitable for the production of a friable tilth without smearing or compaction.

Thus, workability does not define a precise soil condition because satisfactory operations depend on the operator and machinery as well as the various responses to tillage of different soil types. For example, seed-bed preparation on a loamy soil usually produces a more friable tilth than the same operation on a sand or clay. As with trafficability, workability implies that soil structural damage is avoided. The period of time when land is considered workable is expressed by the term 'machinery work-days' (Rounsevell and Jones 1993).

The soil factors considered are wetness class, which quantifies the depth and duration of waterlogging in the soil profile, and retained water capacity, which is a function of texture, organic matter content and bulk density. These are used to assign a workability rating to soils, which reflects their relative suitability for field work. This rating is converted into a number of days that are added to, or subtracted from, the initial climatic estimate of the workable period to derive a final estimate of good machinery work-days (Rounsevell and Jones 1993).

The threshold value for workability is defined as "'the soil moisture status, expressed in moisture content or matric potential, at which tillage is possible with positive effects on soil structure". If the soil is drier than the threshold value tillage activities can be performed without structure deterioration. However, a soil can also be too dry for optimal tillage. The power required for tillage will increase and moreover the operation itself is less effective as the cohesive forces in the soil are too strong for optimal crumbling. Because the latter occurs rarely in the study area, this aspect is excluded (Droogers, Fermont and Bouma, 1996).

The well-known Atterberg test was performed to obtain the lower plastic limit for the three fields. Samples were taken at four randomly chosen plots at each field from the top 20 cm, and the lower plastic limit was determined on I0 replicates for each sample. A drawback of this method could be the subjectivity in the judgement of the state of the plasticity of the sample. To avoid this subjectivity and to evaluate the corresponding error a total of 120

determinations were handled in random order and some determinations were also independently done by two operators (Droogers, Fermont and Bouma, 1996).

The lower plastic limit obtained, expressed as a gravimetric moisture content, was converted to volumetric moisture content by multiplying it by the bulk densities of the samples.

The threshold value for trafficability, also expressed in moisture content or matric potential, is the soil moisture status at which "'soil traffic is possible without causing unfavourable compaction". Penetrometer measurements are useful for determining the trafficability of a field. For the three fields with the different management types, the relation between soil moisture content and penetration resistance was obtained. By combining this relation with a critical penetration resistance for trafficability, the threshold value for trafficability was estimated for the three fields. Critical penetration resistances for trafficability can be found in literature (e.g. Rounsevell, 1993). However, values are obtained with a large diversity of penetrometers and measurement techniques (Droogers, Fermont and Bouma, 1996).

Upper workability limit (UWL) can be defined as the upper limit of soil moisture when tillage operation is performed.For tillage operations purposes, this limit is sometimes called the upper tillage limit (UTL), wet workability limit (WWT) or moisture threshold and workability threshold.

Lower workability limit (LWL) can be defined as the lower limit of soil moisture when tillage operation is performed, often called as the lower tillage limit (LTL) and dry workability limit (DWL).

The optimum water content for tillage (OPT) can be defined as "the water content at which tillage produces the greatest proportion of small aggregates". If soil is tilled when it is wetter than this optimum water content, then large clods can be produced and soil structural damage can occur. If the soil is drier than the optimum water content, then tillage requires excessive energy and can also produce large clods. The optimum water content for tillage, θ_{OPT} , was firstly estimated as $0.9\theta_{OPT}$. θ_{OPT} is very close to the water content at the inflection point, θ_{INFL} . Therefore, it was decided to adopt the relationship (Dexter and Bird, 2001).

$\theta_{OPT} = \theta_{INFL}$, (2)

The range of water contents, $\Delta\theta_{RANGE}$, over which tillage may satisfactorily be done is defined as the difference between UTL and LTL :

$\Delta\theta_{RANGE} = \theta_{UTL} - \theta_{LTL}$, (3)

Various degrees of soil moisture content is assumed for UWL but the most common is the (lower) plastic limit (PL) (de Toro and Hansson, 2004) and usually 0.9 PL is assigned as the optimum water content for tillage (Dexter and Bird, 2001).

There are two methods for determining probability of a working day as follow. Use of actual data of workdays: In this method the workability condition of soils in each region is studied for several years, thus, reliable results will be gained from the of the study and the results are only useful for the condition that study is conducted and can hardly be attributed to other conditions. So, for different types of soils, regions and operating conditions, separate studies are required. Edwards and Boehlje (1980) used this method for determination of probability of a working day in USA. Use of soil water balance models: In this method, soil moisture and its workability can be estimated by means of historical weather data and soil hydraulic characteristics, in previous years. Simalenga and Have (1992) in Tanzania, Rounsevell and Jones (1993) in England, Droogers et al. (1996) in the Netherlands, Cooper et al. (1997) in

Scotland and Rotz and Harrigan (2005) in USA have used this method for determination of probability of a working day.

This study was planned to develop and verify a model using meteorological data and soil characteristic that can efficiently determine the probability of a workday suitable for tillage operations in different region by different weather and soil characteristics. Finally, the sensitivity analysis of the model was applied to the main inputs to determine the needed accuracy of these inputs for precise application of the model.

MATERIALS AND METHODS

In this model, the soil profile is defined as having layers for which workability limits can be assigned by user. Each layer is subdivided into several depth compartments. The size of each depth increment and the length of the time interval is determined by user. By decreasing the size of depth increment and the time interval, accuracy of simulation increases but in turn the time length of the simulation increases.

Halfway within each soil compartment a node is defined,the soil profile is therefore divided into a number of compartments of same specified thickness and the total time period into discrete time increments (time steps). Fluxes through these divisions are calculated to estimate changes in the soil water content. Fluxes are considered to be constant during an individual time step.

By dividing the soil profile into several depth increments, moisture transfer between layers (depth increments) at different time steps is computed and by summing the net input water to moisture content at previous time step at each layer, new moisture content is calculated. This cycle is continued until soil water content of each layer at all-time steps of a day is determined. Soil moisture at each layer in each day is the average of soil moisture at time steps in daily working hours. The suitability of a given day for tillage operation is determined by comparing the moisture of the upper soil layers for which tillage operation is influenced by. The moisture level in the remainder of the soil profile does not directly affect workability. By adding up the workable days, number of workdays is determined. The basic flowchart of the model is represented in figure 1.

Water balance for the surface of soil

Evaporation is the process whereby liquid water is converted to water vapour (vaporization) and removed from the evaporating surface (vapour removal). As evaporation proceeds, the surrounding air becomes gradually saturated and the process will slow down and might stop if the wet air is not transferred to the atmosphere. The replacement of the saturated air with drier air depends greatly on wind speed. Hence, solar radiation, air temperature, air humidity and wind speed are climatological parameters to consider when assessing the evaporation process.

Soil evaporation from the exposed soil can be assumed to take place in two stages: an energy limiting stage, and a falling rate stage. When the soil surface is wet, K_r is 1. When the water content in the upper soil becomes limiting, K_r decreases and becomes zero when the total amount of water that can be evaporated from the topsoil is depleted (figure 2).

In the simple evaporation procedure, it is assumed that the water content of the evaporating layer of the soil is at field capacity (θ_{FC}), shortly following a major wetting event and that the soil can dry to a soil water content level that is halfway between oven dry (no water left) and wilting point (θ_{WP}).The amount of water that can be depleted by evaporation during a complete drying cycle can hence be estimated as:

$$TEW = 1000(\theta_{FC} - 0.5\theta_{WP})Z_e \quad (4)$$

where TEW total evaporable water = maximum depth of water that can be evaporated from the soil when the topsoil has been initially completely wetted [mm],

θ_{FC} soil water content at field capacity [m3 m-3],

θ_{WP} soil water content at wilting point [m3 m-3],

Z_e depth of the surface soil layer that is subject to drying by way of evaporation [0.10-0.15 m].

Where unknown, a value for Z_e, the effective depth of the soil evaporation layer, of 0.10-0.15 m is recommended.

Stage 1: energy limiting stage

At the start of a drying cycle, following heavy rain or irrigation, the soil water content in the topsoil is at field capacity and the amount of water depleted by evaporation (D_e), is zero. During stage 1 of the drying process, the soil surface remains wet and it is assumed that evaporation from soil exposed to the atmosphere will occur at the maximum rate limited only by energy availability at the soil surface. This stage holds until the cumulative depth of evaporation (D_e), is such that the hydraulic properties of the upper soil become limiting and water cannot be transported to the soil surface at a rate that can supply the potential demand. During stage 1 drying, $K_r = 1$.

The cumulative depth of evaporation (D_e), at the end of stage 1 drying is REW (Readily evaporable water, which is the maximum depth of water that can be evaporated from the topsoil layer without restriction during stage1). The depth normally ranges from 5 to 12 mm and is generally highest for medium and fine textured soils.

Stage 2: falling rate stage

The second stage (where the evaporation rate is reducing) is termed the 'falling rate stage' evaporation and starts when D_e exceeds REW. At this point, the soil surface is visibly dry, and the evaporation from the exposed soil decreases in proportion to the amount of water remaining in the surface soil layer:

$$K_r = \frac{TEW - D_{e,i-1}}{TEW - REW} \qquad D_{e,i-1} \succ \text{REW} \quad (5)$$

where K_r dimensionless evaporation reduction coefficient dependent on the soil water depletion (cumulative depth of evaporation) from the topsoil layer ($K_r = 1$ when $D_{e,i-1} \leq \text{REW}$),

$D_{e,i-1}$ cumulative depth of evaporation (depletion) from the soil surface layer at the end of day_{i-1} (the previous day) [mm],

TEW maximum cumulative depth of evaporation (depletion) from the soil surface layer when $K_r = 0$ (TEW = total evaporable water) [mm],

REW cumulative depth of evaporation (depletion) at the end of stage 1 (REW = readily evaporable water) [mm].

daily water balance computation for the surface soil layer :
The daily soil water balance equation for the exposed and wetted soil fraction few is (figure 3):

$$D_{e,i} = D_{e,i-1} - (P_i - RO_i) + \frac{E_i}{f_{ew}} + DP_{e,i} \quad (6)$$

where $D_{e,i-1}$ cumulative depth of evaporation following complete wetting from the exposed and wetted fraction of the topsoil at the end of day_{i-1} [mm],

$D_{e,i}$ cumulative depth of evaporation (depletion) following complete wetting at the end of day_i [mm],

P_i precipitation on day_i [mm],

RO_i precipitation runoff from the soil surface on day_i [mm],

E_i evaporation on day_i [mm],

f_{ew} exposed and wetted soil fraction [0.01 - 1].

$DP_{e,i}$ deep percolation loss from the topsoil layer on day_i if soil water content exceeds field capacity [mm],

Limits on $D_{e,i}$

By assuming that the topsoil is at field capacity following heavy rain or irrigation, the minimum value for the depletion $D_{e,i}$ is zero. As the soil surface dries, $D_{e,i}$ increases and in absence of any wetting event will steadily reach its maximum value TEW (Equation 4). At that moment no water is left for evaporation in the upper soil layer, K_r becomes zero, and the value for $D_{e,i}$ remains at TEW until the topsoil is wetted once again. The limits imposed on $D_{e,i}$ are consequently:

$$0 \le D_{e,i} \le \text{TEW} \quad (7)$$

Initial depletion

To initiate the water balance for the evaporating layer, the user can assume that the topsoil is near field capacity following a heavy rain or irrigation, i.e., $D_{e,i} = 0$. Where a long period of time has elapsed since the last wetting, the user can assume that all evaporable water has been depleted from the evaporation layer at the beginning of calculations, i.e., $D_{e,i-1} = TEW = 1000(\theta_{FC} - 0.5\theta_{WP})Z_e$.

Precipitation and runoff

P_i is equivalent to daily precipitation. Daily precipitation in amounts less than about $0.2ET_o$ is normally entirely evaporated and can usually be ignored in the K_e and water balance calculations. The amount of rainfall lost by runoff depends on: the intensity of rainfall; the slope of land; the soil type, its hydraulic conditions and antecedent moisture content; and the land use and cover. For general situations, RO_i can be assumed to be zero or can be

accounted for by considering only a certain percentage of P_i. This is especially true for the water balance of the topsoil layer, since almost all precipitation events that would have intensities or depths large enough to cause runoff would probably replenish the water content of the topsoil layer to field capacity. Therefore, the impact of the runoff component can be ignored. Light precipitation events will generally have little or no runoff. In this study, surface runoff is estimated using the SCS Runoff Curve Number (CN) method developed by U.S. Soil Conservation Service (SCS) in 1972. The SCS runoff equation is:

$$Q = \frac{(P - I_a)^2}{P - I_a + S} \quad \text{for} \quad \mathrm{P} \succ I_a$$
$$Q = 0 \quad \text{for} \quad \mathrm{P} \le I_a \qquad (8)$$

Where P is the total rainfall, I_a is the initial abstraction, Q is the direct runoff and S is the potential maximum retention. Based on a second assumption, that the amount of initial abstraction is a fraction of the potential maximum retention, $I_a = \lambda S$, Eq. (9) becomes :

$$Q = \frac{(P - \lambda S)^2}{P + (1 - \lambda)S} \Rightarrow \text{if } \lambda = 0.2 \Rightarrow Q = \frac{(P - 0.2S)^2}{P + 0.8S} \qquad (9)$$

The potential retention S is expressed in terms of the dimensionless curve number (CN) through the relationship. In the SI units (with S in mm) the following definition should be used:

$$S = \frac{25400}{C.N} - 254 \quad , (10)$$

The runoff curve number (CN), which depends on soil characteristics, vegetativecover, and hydrological conditions, is provided by SCS (1986). More than 4,000 soils have been classified by the SCS (1986) into four hydrological soil groups. The four hydrologic soil groups are classified as A, B, C, and D.

Evaporation

Evaporation beneath the vegetation canopy is assumed to be included in K_{cb} and is therefore not explicitly quantified. The computed evaporation is fully concentrated in the exposed, wetted topsoil. The evaporation E_i is given by $K_e ET_o$. The E_i / f_{ew} provides for the actual concentration of the evaporation over the fraction of the soil that is both exposed and wetted.

Deep percolation

Following heavy rain or irrigation, the soil water content in the topsoil (Z_e layer) might exceed field capacity. However, in this simple procedure it is assumed that the soil water content is at θ_{FC} nearly immediately following a complete wetting event, so that the depletion $D_{e,i}$ in Equation 6 is zero. Following heavy rain or irrigation, downward drainage (percolation) of water from the topsoil layer is calculated as:

$$DP_{e,i} = (P_i - RO_i) - D_{e,i-1} \geq 0 \quad (11)$$

As long as the soil water content in the evaporation layer is below field capacity (i.e., $D_{e,i} \succ 0$), the soil will not drain and $DP_{e,i} = 0$.

2: water balance for tillage zone

It makes the adding and subtracting of losses and gains straightforward as the various parameters of the soil water budget are usually expressed in terms of water depth. Soil evaporation and percolation losses remove water from the increase the depletion. The daily water balance for tillage zone, expressed in terms of depletion at the end of the day is:

$$D_{r,i} = D_{r,i-1} - (P_i - RO_i) + E_i + DP_i - CR_i \quad (12)$$

where $D_{r,i}$ Tillage zone depletion at the end of day_i [mm],

$D_{r,i-1}$ Water content in the tillage zone at the end of the previous day_{i-1} [mm],

DP_i water loss out of the zone by deep percolation on day_i [mm].

CR_i Capillary rise from the groundwater table on day_i [mm],

Limits on $D_{r,i}$

In Figure 4 it is assumed that water can be stored in the soil zone until field capacity is reached. Although following heavy rain or irrigation the water content might temporally exceed field capacity, the total amount of water above field capacity is assumed to be lost the same day by deep percolation, following any evaporation for that day. By assuming that the soil zone is at field capacity following heavy rain or irrigation, the minimum value for the depletion $D_{r,i}$ is zero. As a result of percolation and evapotranspiration, the water content in the tillage zone will gradually decrease and the tillage zone depletion will increase. In the absence of any wetting event, the water content will steadily reach its minimum value θ_{WP}. At that moment no water is left for evaporation in the tillage zone, K_r becomes zero, and the tillage zone (tillage zone) depletion has reached its maximum value TEW. The limits imposed on $D_{r,i}$ are consequently:

$$0 \leq D_{r,i} \leq TEW \quad (13)$$

Initial depletion

To initiate the water balance for the tillage zone, the initial depletion $D_{r,i-1}$ should be estimated. The initial depletion can be derived from measured soil water content by:

$$D_{r,i-1} = 1000(\theta_{FC} - \theta_{i-1})Z_r \quad (14)$$

where θ_{i-1} is the average soil water content for the effective tillage zone. Following heavy rain or irrigation, the user can assume that the tillage zone is near field capacity, i.e., $D_{r,i-1} \approx 0$.

Deep percolation (DP)

Following heavy rain or irrigation, the soil water content might exceed field capacity. In this simple procedure it is assumed that the soil water content is at θ_{FC} within the same day of the wetting event, so that the depletion $D_{r,i}$ in Equation 12 becomes zero.Therefore, following heavy rain or irrigation

$$DP_i = (P_i - RO_i) - E_i - D_{i,i-1} \geq 0 \quad (15)$$

As long as the soil water content in the tillage zone is below field capacity (i.e., $D_{r,i} \succ 0$), the soil will not drain and $DP_i = 0$.

Capillary rise (CR)

The amount of water transported upwards by capillary rise from the water table to the tillage zone depends on the soil type, the depth of the water table and the wetness of the tillage zone. CR can normally be assumed to be zero when the water table is more than about 1 m below the bottom of the tillage zone.

Calculating evaporation

The point at which the second stage of evaporation begins is called threshold point. Potential evaporation (stage one evaporation) is computed by Penman-Monteith equation (Monteith, 1965):

$$E_p = \frac{\Delta(R_n - G_s) + 86.4\rho\, c_p \delta / r_a}{\lambda(\Delta + \gamma)} \quad (16)$$

where E_p is the potential soil evaporation ($kgm^{-2}d^{-1} \approx mmd^{-1}$); Δ, the slope of saturated vapor pressure–temperature curve ($kPa^{\circ}C^{-1}$); R_n, the net radiation ($MJm^{-2}d^{-1}$); G_s, the soil heat flux ($MJm^{-2}d^{-1}$); ρ, the air density (kgm^{-3}); c_p, the specific heat of air ($kJkg^{-1}C^{-1} = 1.013$); δ, the vapor pressure deficit of the air (kPa); r_a, the aerodynamic resistance (sm^{-1}); λ, the latent heat of vaporization ($MJkg^{-1}$); γ, the psychrometric constant ($kPa^{\circ}C^{-1}$), and; 86.4, the factor for conversion from kJs^{-1} to MJd^{-1}.

The model used to estimate actual evaporation from bare soils is based on approach originally proposed by Aydin et al. (2005) equation:

$$E_a = \frac{1}{\log|\psi_{tp}| - \log|\psi_{ad}|}(\log|\psi| - \log|\psi_{ad}|)E_p \quad (17)$$

Where : E_a and E_p are actual and potential evaporation rates, respectively; ψ_{tp}, the absolute values of soil–water potential (matric potential) at which actual evaporation starts to drop below potential one; ψ_{ad}, the absolute values

of soil–water potential at air-dryness, and; ψ is the absolute value of soil–water potential to be determined in situ between ψ_{tp} and ψ_{ad}. The values of all ψ are in cm of water.

Assuming that the water potential at dry soil surface is at equilibrium with the atmosphere, the minimum water potential can be derived from the Kelvin equation (Brown and Oosterhuis, 1992; Feddes et al.,1978; Kirby and Ringrose-Voase, 2000.

$$\psi_{ad} = \frac{R_g T}{mg} \ln H_r \quad (18)$$

Where ψ_{ad} is the water potential for air-dry conditions (cm of water), T, the absolute temperature (K), g, the acceleration due to gravity (981 $cm\,s^{-2}$); m, the molecular weight of water ($0.01802\ \mathrm{kg\ mol^{-1}}$), H_r, the relative humidity of the air (fraction), and; R_g, the universal gas constant ($8.3143\times10^{4}\ kg\ \mathrm{cm^2\ s^{-2}\ mol^{-1}\ K^{-1}}$).

Calculate the moisture transfer :

In this model Soil-water flow is simulated using the Richard's equation. This equation is derived from combining the continuity equation with Darcy's Law. For vertical one-dimensional flow under transient conditions this equation is :

$$\frac{\partial\theta}{\partial t} = \frac{\partial}{\partial z}\left[K(\theta)\left(\frac{\partial h}{\partial z}+1\right)\right] \quad (19)$$

where: h is the water pressure head [L], θ is the volumetric water content [L^3L^{-3}], t is time [T], z is the spatial coordinate [L] (positive upward) and K is the unsaturated hydraulic conductivity function [LT^{-1}].

This equation is solved by numerical finite difference method. The model uses the explicit method in which it is assumed that matric potential and hydraulic conductivity, thus, water flow between two depth increments in a time step are constant. Also, it is assumed that a depth increment has uniform hydraulic characteristic:

$$\frac{\theta_i^{j+1,k+1}-\theta_i^{j}}{\Delta t} = \frac{1}{\Delta z}\left[K_{i+1/2}^{j+1,k}\frac{h_{i+1}^{j+1,k+1}}{\Delta z_i} - K_{i-1/2}^{j+1,k}\frac{h_i^{j+1,k}-h_{i+1}^{j+1,k+1}}{\Delta z_{i-1}}\right] + \frac{K_{i+1/2}^{j+1,k}-K_{i-1/2}^{j+1,k}}{\Delta z} \quad (20)$$

Where :

$$\Delta t = t^{j+1} - t^{j} \quad , (21)$$

$$\Delta z = \frac{z_{i+1}-z_{i-1}}{2} \qquad \Delta z_i = z_{i+1}-z_i \qquad \Delta z_{i-1} = z_i - z_{i-1} \quad , (22)$$

$$K_{i+1/2}^{j+1,k} = \frac{K_{i+1}^{j+1,k}+K_i^{j+1,k}}{2} \qquad K_{i-1/2}^{j+1,k} = \frac{K_i^{j+1,k}+K_{i-1}^{j+1,k}}{2} \quad (23)$$

in which subscripts $i-1$, i, and $i+1$ indicate the position in the finite difference mesh; superscripts k and $k+1$ denote the previous and current iteration levels, respectively; and superscripts j and $j+1$ represent the previous and current time levels, respectively. Equation (20) is based on a fully implicit discretization of the time derivative, and will be solved with a Picard iterative solution scheme. The mass-conservative method proposed by Celia et al. [1990], in which $\theta^{j+1,k+1}$ is expanded in a truncated Taylor series with respect to h about the expansion point $h^{j+1,k}$, is used in the time difference scheme of (24):

$$\frac{\theta_i^{j+1,k+1}-\theta_i^{j}}{\Delta t}=C_i^{j+1,k}\frac{h_i^{j+1,k+1}-h_i^{j+1,k1}}{\Delta t}+\frac{\theta_i^{j+1,k}-\theta_i^{j}}{\Delta t} \quad (24)$$

where C_i represents the nodal value of the soil water capacity [L^{-1}]:

$$C_i^{j+1,k}=\left.\frac{d\theta}{dh}\right|^{j+1,k} \quad (25)$$

For solution of this equation, hydraulic conductivity and soil water potential functions must be known. Van Genutchen equation (1980) was used for determination of soil water suction:
The van Genuchten – Mualem expression for the water retention curve $\theta(h)$ is:

$$\theta(h)=\theta_r+(\theta_s-\theta_r)\Big/\left(1+(\alpha h)^n\right)^m \quad (26)$$

where θ_r is the residual water content ($cm^3\ \mathrm{cm}^{-3}$), θ_s is the saturated water content($cm^3\ \mathrm{cm}^{-3}$) , and α (m^{-1}), n , and m are empirical parameters . When fitting $\theta(h)$ data independently, the assumption $m=1-1/\mathrm{n}$, $(\mathrm{n}\succ 1)$ is used for Eq. (26).

Unsaturated hydraulic conductivity ($m\,s^{-1}$)is determined from Van Genutchen equation (1980) :

$$K(h)=K_s\times S_e^L[1-(1-S_e^{1/m})^m]^2 \quad (27)$$

where K_s is the saturated hydraulic conductivity ($m\,s^{-1}$) , L is a factor that accounts for the pore connectivity and tortuosity estimated by Mualem to be 0.5 as an average of many soils. S_e the relative saturation defined as:

$$S_e=\frac{(\theta-\theta_r)}{(\theta_s-\theta_r)} \quad (28)$$

Investigation of tillage possibility:

Given the amount of potential evaporation, evaporation, runoff and rainfall, soil moisture were measured at different layers, if the humidity is greater than field capacity in last layer, remaining water to penetrate into the lower parts. The moisture of each layer compared with the moisture which determined by user. For a day must be introduced as a day of workday should in all layers within the range of moisture content of the layer is simulated. The moisture should not be lower and upper than workability limit. If all these conditions are met that day is known as a workday, otherwise it will not suitable for workday. After summing suitable workday and dividing to total duration of the simulation period (season) probability of workday in that period is obtained.

RESULTS AND DISCUSSION

Validation of the model in estimating soil moisture:

It is necessary to check the validity of the model results to be compared with actual measurements. For the simulation model inputs should be measured in accordance with the conditions to be able to compare the model

results with measured data. For this purpose, three field tests were conducted 3 cities in the province of Qazvin in Iran (Qazvin - Abyek and Bouin Zahra). In this test inputs mesured by Time Domain Reflectometry (TDR) in 0-25 and 0-30 cm depths. For a period of 31 days (From 21 March up to 20 April 2014) the TDR were read 3 times a day (6 am, 12 am and 6 pm) Regarding this fact that there were three farms, two probs in each farms, three times data collection in each days and 31 days in verification periods. Statistical and graphical methods were used for this purpose. the Mean Bias Error, Coefficient of Variation, Modeling Efficiency, Mean Relative Percentage Deviation, Regression Sum of Squares Squares and Coefficient of Determination were identified as key indicators of model validation. Indicators been mentioned for Abyek city of Qazvin and Buin Zahra comparison chart with estimated soil moisture content and soil moisture content measurements in charts 1, 2 and 3 are shown.

As shown in Table 2, the measured and estimated the moisture in most areas are very close together. The mean value and standard deviation (MBE) in cities Buin Zahra and Abyek represents low models, and the city of Qazvin, indicating overestimation model. The total value of the deviation of the observed values are very low. Note that this value is close to zero, indicating good estimation model and can be seen very little deviation.
Note that the percent coefficient of variation (CV) is lower in the cities studied showed high accuracy in estimating the soil moisture.

The Modeling Efficiency (ME) represents the observed data and estimate the quality of the fit between the -0.12 to -0.47 variable. Due to its proximity to the Modeling Efficiency values of 1, optimal fit between measured and estimated values of the moisture there, The result shows the accuracy of the model is to estimate soil the moisture.
The results of the calculation of the Mean Relative Percentage Deviation (MRPD) Show an acceptable fit to the data model and the model can be used to measure the moisture content of the soil.

The regression sum of squares of squares (SSE) in the fitting process must be as low as possible, because it represents the amount of random errors and the quantity is less, will increase the accuracy of the model, the results show the accuracy of the model is to SSE .
The coefficient of determination (R^2) in model cities studied, from 0.81 to 0.88, which represents the probability of correlation between the two data sets in the future. Approximately 81 to 88 percent of the dispersion between the estimated and measured values are shared.

Validation of the model to simulate the moisture release curve and unsaturated hydraulic conductivity:

The number of variables that must be estimated moisture curve fitting process five $n, \theta_r, \theta_s, \alpha$ and m respectively. for determining unsaturated hydraulic conductivity in addition to the above factors can be calculated in two variables K_S and L in the model. The number of potential variables in determining soil moisture release curve and hydraulic functions are seven factors that can be designed into a single model, the fitting.
To ensure that the results of the model, all variables with the values obtained;
a: computer program Rosetta in 2001 by the Schaap and al. developed in the laboratory of soil salinity America,
b: database Rawls et al in 1982,
c: database provided Carsel and al in 1988.
Were compared (see table 3 , 4 and 5).

To greater certainty the estimation of the model, Ratio the amount calculated based on the observations (R') to check that the results obtained (R'), it becomes clear that the residual water content (θ_r), water content at saturation (θ_s), reverse air entry potential soil (α), determines the shape of the curve is an empirical coefficient (n), saturated hydraulic conductivity (K_S) and experimental parameters (distortion factor) (L)calculated in comparison with the database, which is close to the prediction accuracy of the model parameters is indicated.

Probability of of workdays

The probability of workdays, depending on the conditions of the ability to do the following actions were:
first condition: Delimitation feature work by taking the upper limit and lower limit of the workability .
Second condition: Delimitation feature work by considering only the upper limit of the workability.

As the results shown in Table 6 If the lower limit workability is not considered all of days can be considered as workday. The most probability of workday was the used formula by Department of Soil America, because the low limit workability was the lowest. the lest probability of workday was the used formula by Dexter and Bird.

As seen in Table 6, when just the upper limit workability as tillage limiting factor be considered, a relatively large probability obtained workday. It seems that this is because of the hot and arid climate due to low rainfall and high evaporation in tillage season, soil moisture rarely goes above the upper limit workability. Even a small reduction in the value of this limit, it would be great workdays. The result can be considered upper limit workability lower than last and tillage operations performed in the near optimum moisture content.

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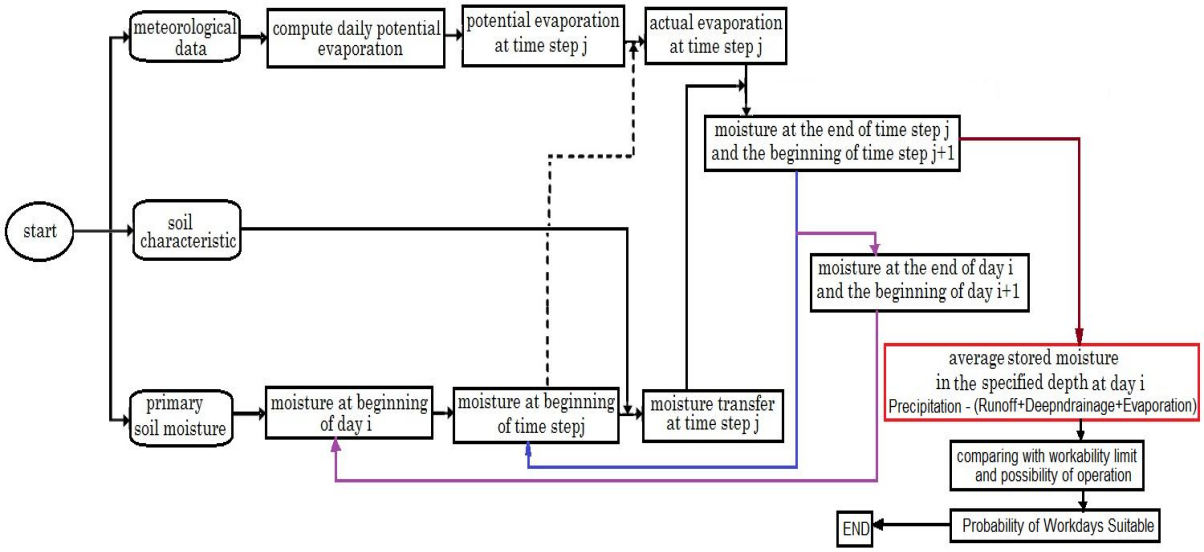

Figure1: Basic flowchart of the model

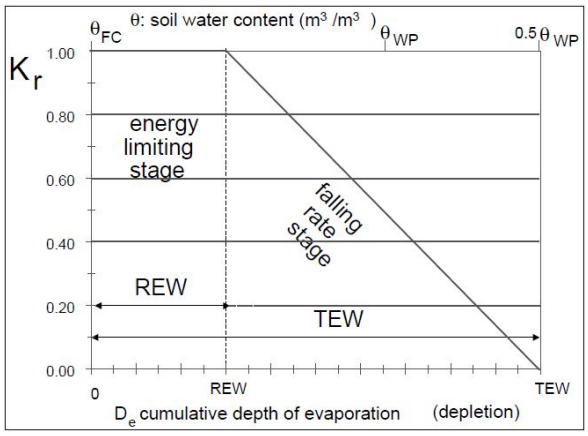

Figure2: Soil evaporation reduction coefficient, K_r

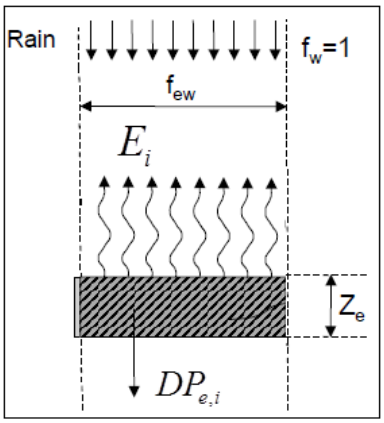

Figure3: Water balance of the topsoil layer

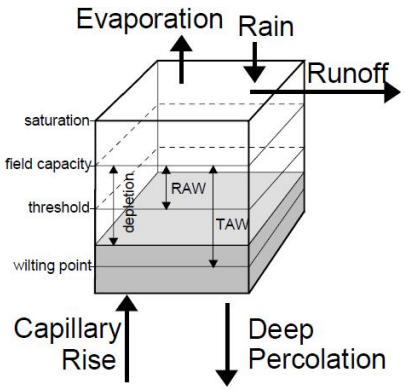

Figure4: Water balance of the tillage zone

Chart 1. Comparison of estimated and measured moisture in Bouin Zahra(From 21 March up to 20 April 2014)

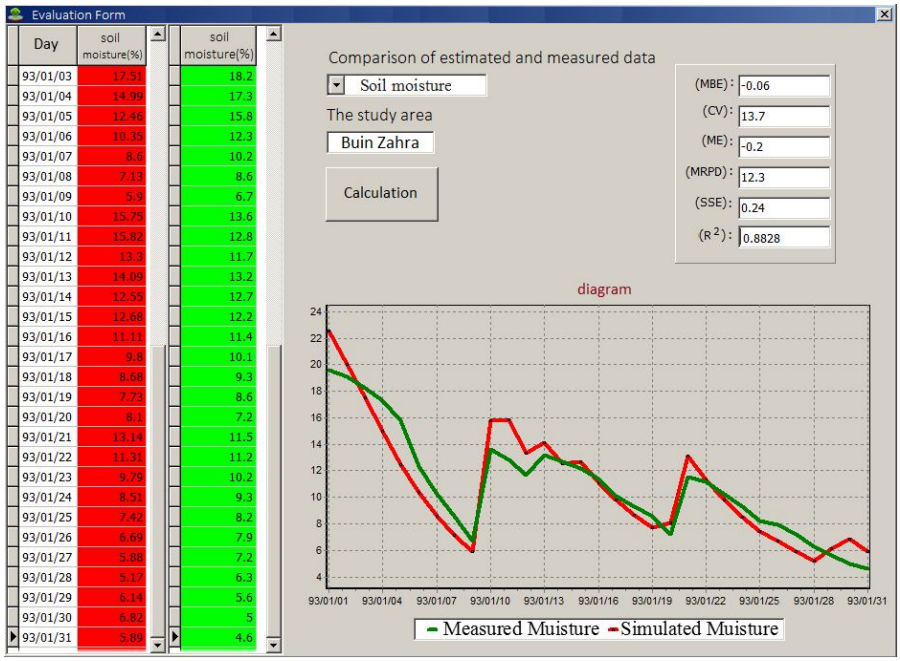

Chart 2. Comparison of estimated and measured moisture in Abyek(From 21 March up to 20 April 2014)

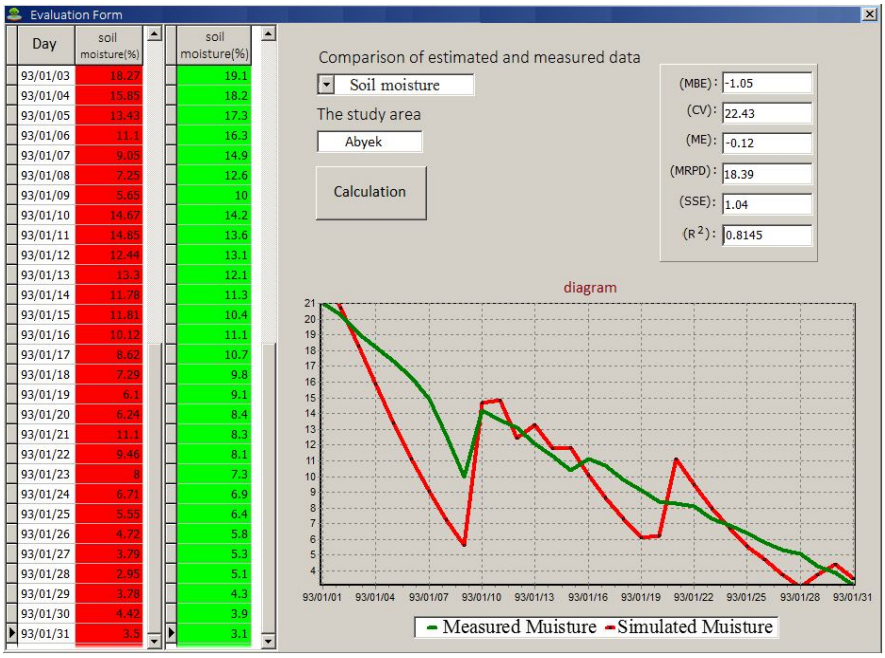

Chart 3. Comparison of estimated and measured moisture in Qazvin(From 21 March up to 20 April 2014)

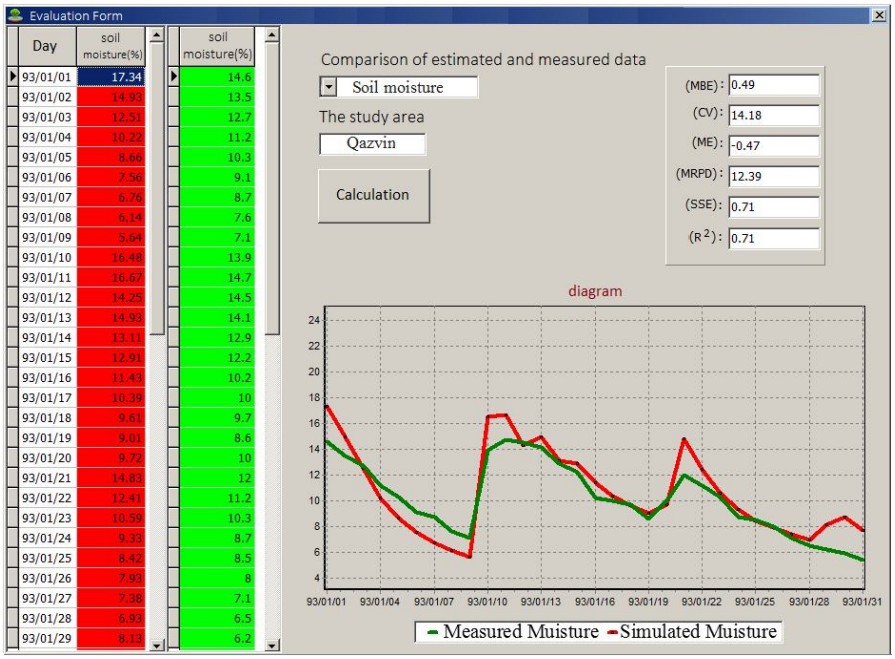

Table 1: different parts of designed model

Log in Information	Information Processing	Charts	Validation
1. The registration of geographical location	1. Calculate the potential evaporation	1. Weather information	Evaluation form 1
	2. Calculation of soil moisture characteristic curve		
2. The data recording area , crop and tillage	3. Calculate the unsaturated hydraulic conductivity curve	2. The moisture transfer calculations	
	4. Calculation of runoff		Evaluation form 2
3. Weather information	5. The calculation of evaporation		
	6. Calculation of workability limit	3. determination of workdays	
4. Information of soil characteristics	7. Calculation of possibility tillage		
	8. A applicable example : the number of machine		

Table 2: Comparison of estimated and measured moisture

City	MBE	CV	ME	MRPD	SSE	R^2
Buin Zahra	-0.06	13.7	-0.2	12.3	0.24	0.88
Abyek	-1.05	22.43	-0.12	18.39	1.04	0.81
Qazvin	0.49	14.18	-0.47	12.39	0.71	0.83

Table 3: Evaluation of soil moisture characteristic curve and unsaturated hydraulic conductivity estimated and presented in Bouin Zahra

Evaluation the Moisture release curve and unsaturated hydraulic conductivity

The study area	Texture	θ_r	θ_s	α	n	K_S	L
Bouin Zahra	Loam	0.0718	0.4637	0.029	1.1988	9.93072	-0.4484
Database	**Texture**	θ_r	θ_s	α	n	K_S	L
Rosetta (Schaap and al.)	Loam	0.061	0.399	0.011	1.4723	12.05	-0.371
Rawls and al.	Loam	0.027	0.434	0.09	1.22	16.32	0.5
Carsel and al.	Loam	0.078	0.43	0.036	1.56	24.96	0.5

Ratio the amount estimated by the model to the measured value

Rosetta (Schaap and al.)	1.177	1.162	2.635	0.814	0.824	1.209
Rawls and al.	2.658	1.069	0.322	0.983	0.609	-0.897
Carsel and al.	0.92	1.078	0.805	0.768	0.398	-0.897

Calculation

Table 4: Evaluation of soil moisture characteristic curve and unsaturated hydraulic conductivity estimated and presented in Abyek

Evaluation the Moisture release curve and unsaturated hydraulic conductivity

The study area	Texture	θ_r	θ_s	α	n	K_S	L
Abyek	Clay Loam	0.0674	0.4636	0.0419	1.1664	31.4339	-0.6944
Database	**Texture**	θ_r	θ_s	α	n	K_S	L
Rosetta (Schaap and al.)	Clay Loam	0.079	0.442	0.0158	1.4157	8.184	-0.763
Rawls and al.	Clay Loam	0.075	0.39	0.039	1.194	5.52	0.5
Carsel and al.	Clay Loam	0.095	0.41	0.019	1.31	6.24	0.5

Ratio the amount estimated by the model to the measured value

Rosetta (Schaap and al.)	0.853	1.049	2.654	0.824	3.841	0.91
Rawls and al.	0.898	1.189	1.075	0.977	5.695	-1.389
Carsel and al.	0.709	1.131	2.207	0.89	5.037	-1.389

Calculation

Table 5: Evaluation of soil moisture characteristic curve and unsaturated hydraulic conductivity estimated and presented in Qazvin

Evaluation the Moisture release curve and unsaturated hydraulic conductivity

The study area	Texture	θ_r	θ_s	α	n	K_S	L
Qazvin	Sandy Loam	0.0777	0.4074	0.0461	1.2589	23.87801	-0.415

Database	Texture	θ_r	θ_s	α	n	K_S	L
Rosetta (Schaap and al.)	Sandy Loam	0.039	0.387	0.0266	1.4487	38.282	-0.861
Rawls and al.	Sandy Loam	0.041	0.412	0.068	1.322	62.16	0.5
Carsel and al.	Sandy Loam	0.065	0.41	0.075	1.89	106.1	0.5

Ratio the amount estimated by the model to the measured value

Rosetta (Schaap and al.)	1.991	1.053	1.734	0.869	0.624	0.482
Rawls and al.	1.894	0.989	0.678	0.952	0.384	-0.83
Carsel and al.	1.195	0.994	0.615	0.666	0.225	-0.83

Calculation

Table 6: The results probability of workdays according to the conditions of investigated workability

The used formula	Period		Area	workability limit			Soil texture	Case study	The number of workdays	probability of workdays
	Start	end		Down	Desired	up				
Department of Soil America	2014/03/21	2014/04/20	Qazvin	13.51	17.58	25.84	Sandy Loam	1	7	22.5%
								2	31	100%
			Buin Zahra	14.55	15.64	36.82	Loam	1	8	25.8%
								2	31	100%
			Abyek	17.91	18.1	40.61	Clay Loam	1	3	9.7%
								2	31	100%
Mueller - Schindler	2014/03/21	2014/04/20	Qazvin	14.81	18.68	21.46	Sandy Loam	1	6	19.3%
								2	31	100%
			Buin Zahra	20.13	27.21	32.7	Loam	1	2	6.4%
								2	31	100%
			Abyek	19.47	19.85	43.24	Clay Loam	1	2	6.4%
								2	31	100%
Dexter and Bird	2014/03/21	2014/04/20	Qazvin	21.16	23	25	Sandy Loam	1	0	0
								2	31	100%
			Buin Zahra	26.16	28.44	30.91	Loam	1	0	0
								2	31	100%
			Abyek	27.15	29.51	32.08	Clay Loam	1	0	0
								2	31	100%

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RESEARCH ARTICLE

Evaluating Economical – Social Effects of Regional-National Projects of Sistan

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ABSTRACT

Regional-national projects are of special importance in economic and budgeting system of the country and also devote main part of the budget of country. In this regard, the most important cause of performing the present research is being unknown the developmental effects of the actions and regional politics in Sistan. In this project, all effects of performed chosen projects as water transfer project form Sistan to Zahedan, Sistan drainage, half fourth well, transit road, cement plant, boundary wall, silk bridge, space organization change of the region from one city to three cities, changing rural residence to cities and developing higher education factory have been evaluated in Sistan during ten years ago. Moreover, the research has included developmental purpose and it has been done by descriptive-analytical method which most of its data has been collected based on field studies and by sampling method (Cochran) of 124 official and nonofficial experts of the sample area. Also, it has been used the comparative tests (Mann-Whitney) and Analytical Hierarchy procedure (AHP) in order to analyze the data. Research findings based on the results of Mann- Whitney comparative tests showed that there isn't significant relation in Sistan area during the years (2000-2010) between official and nonofficial experts' views on the effect of employment of performed projects. According to research findings, strategies were suggested like preparing conditions toward constant local development relying on local capitals and increasing the residents' role of the area in developing the area, expert's volunteer participation at performing the research, using possibilities, equipments and local human force which not only causes preparing employment but also causes kind of compassion in protecting and keeping the research among local people and things like that.

Key words: evaluation, economical-social effects, regional-national projects, Sistan.

INTRODUCTION

The results of many years centralization-based system, regardless of experts' views and local people in Iran, observed wasting national capitals including physical and spiritual, also wasting governmental possibilities and finally people' dissatisfaction with government and constructive activities, not accomplishing some of construction projects, lack of success and continuance in performing some projects and lack of people's responsibility related to this area (Eftekhari et al. 2011: 62-63). Boundary areas, due to having important role in country and also with regard to including significant volume of population, have special place in planning system of the country and are accounted of main components and elements. Moreover, the government must plan and pay attention to change and develop them in national level. On this base, by performing regional-national projects and plans which have come in different socio-economic development plans, has interfered directly in developing the areas and performs actions in order to achieve the expected goals. It is clear that the governments play effective role in these processes, but playing the role requires factors, backgrounds and possibilities which can have different results in spatial-location conditions and regional features (Saeidi et el. 2009: 48). Today, most development experts believe that it is impossible to achieve constant development and the purposes of construction projects without experts' ideas and people's participation. Doubtlessly, experts' views will facilitate, speed and reduce the cost the development period making and developing plans and regional-national projects at regional-national level and this reality which centralized planning isn't effective and sufficient to achieve development at local level, has been accepted by many countries. So, using official and nonofficial experts' views which are beneficiary and the purpose group of plans and construction projects and provides condition for domination, learning skill and group learning, flexibility in decision-making, mutual notification and consul, increasing people's self-esteem, training active and aware human force for informing and increasing knowledge, increasing society individuals' information and awareness level and finally provides condition to respect people's individual abilities. Also, constructing facilities and installations requires major investment whether the employer be an individual, a private company or governmental organization. Since supplying the resources of such investment is under market demand control or predicted needs, it's expected these facilities achieve and supply some especial goals through specified limitations by employer and related regulations. Everyone knows the importance of experts' and local people's effective role in different stages of preparation and performing regional-national projects and due to their role and their effect of views on plans and projects has been confirmed for advancing the development process. In this regard, the present research is trying to response the question that whether performing regional-national plans and projects in sistan of official and non-official experts' views has been able to cause socio-economic changes and evolutions in the area as an effective factor according to expected purposes in development programs and adopting centralized and up to down approaches in planning process. With respect to suggested problems and issues, achieving the research purposes, the questions ahead are as follows:

1-what is the difference between official and non-official experts' views in ranking the requirements of Sistan area?

2- what is the difference between official and non-official experts' views on the effect of preparing employment of performed actions during the years (2000-2010) in Sistan area?

The research method

In this research, it has been used two methods descriptive statistic (frequency percent, mean) and analytical method (AHP model and Mann Whitney Test) to analyze data using software SPSS and Expert Choice. Superficial and content validity of questionnaire was done by confirming related experts and its reliability has been estimated

α=0/87 using Cronbach's Alpha Coefficient in this relation, which due to considered thresholds in valid scientific resources has desired reliability. The statistical society includes all geography, social sciences teachers, all mayors and also half of officials and experts of offices and villages of Sistan, which equals 182 people according to statistic in 2011. The sample volume has been specified using Cochran formula at the confidence level 95 percent equals 124 people for analyzing collected data in the form of questionnaire.

Situation of studied area

The studied area is the area of Sistan, the area of Sistan includes three cities Zabol, Zahak and Hirmand in time range of the research (statistics center, 2011). The area has been located in geography range between 30 degrees and 5 minutes till 31 degrees and 28 minutes of geographical latitude with the space of 15197 km and 60 degrees and 15 minutes till 61 degrees and 50 minutes of geographical longitude in southeast of Iran and in the most northern part of Sistan and Balouchestan province which has devoted about 8/1 percent the space of province (Salari Sardari et al. 2009: 3).

RESEARCH FINDINGS

The responders' age range was classified into 5 age groups. 6.5% of the responders were in the age group 15 – 25, 40.3% were in the age group 25 – 35, 44.4% were in the age group 35 – 45, 8.1% were in the age group 45 – 60, and 0.8% of them were in the age group 60 and above. Among 124 participants who were interviewed, 74 people equivalent to 59.7% were males and 50 people equivalent to 40.3% were females. The participants' job status was classified into 7 groups. Among these 7 groups, administrative professionals include 24.2% other people, 20.2% school teachers, 12.1% village coordinators, 11.3% university instructors, 5.6% office managers, and 1.6% mayors.

National – Regional Projects of Sistan

Implementing regional – national plans and projects with definite purposes and policies, have sought to make changes and developments to reach desirable living conditions. In this research, large projects implemented across the region during the past 10 years were considered with regards to regional effects such as the project of water transfer from Sistan to Zahedan, Drainage of Sistan, fourth half well dam, transit road, cement factory, border wall, silk bridge, changing regional space organization from 1 city to 3 cities, changing settlement sites into towns, developing higher education, etc.

CONCLUSION

As shown in table (15), according to the results of the first hypothesis, in the projects of water transfer from Sistan to Zahedan, fourth half well, project of Chabahar – Milak transit road, changing regional space organization from 1 city to 3 cities, developing higher education, changing settlement sites into towns, silk bridge, and border wall no significant difference was seen among the views of administrative and non-administrative experts with respect to the fact that the sig obtained from the indices under investigation was above 0.05 (sig > 0.05). A significant difference in the level of 0.95 was seen among the views of administrative and non-administrative experts only in the case of the two projects of Sistan Plain Drainage and Cement Factory. In the former project, the indices of livelihood and business thriving obtained by Mann Whitney were 1525.000 and 1506.500 respectively and in the latter project, the indices of employment, immigration, hope for survival, and business thriving obtained by Mann Whitney were 1418.500, 1.146, 1447.500, and 1459.000 respectively with the significance level of below 0.05 (Sig < 0.05).

According to Fig. 5, in the overall prioritizing of the projects from the responders' point of view using Expert Choice software, the higher education projects weighing 0.333 had the highest effect among the projects under investigation and the water transfer from Zabol to Zahedan as well as border wall project weighing 0.011 had the lowest effect among the projects under investigation.

Overall prioritizing of the projects

Hypothesis Two

This hypothesis was formed as below:

In the field of the effect of employment, there was a significant difference among the views shared by administrative and non-administrative experts about the actions taken in Sistan region during 2000 to 2010.

The Man Whitney test was used to test this hypothesis with respect to the comparison of the two groups of administrative and non-administrative experts.

According to table (16), the average scores for administrative experts were 629.91 and 612.55 for non-administrative experts.

According to table (17), the measure of Mann Whitney was 189012.500 and the significance level was 0.764. Since the significance level was above 0.05 (Sig > 0.05), this hypothesis was rejected. It means that there was no significant difference among the views shared by administrative and non-administrative experts in the field of the effect of employment through the actions taken in Sistan region during 2000 to 2010.

Suggestions

Given the results obtained from this study, the following suggestions can be proposed in the field of evaluating regional – national projects across Sistan region:

Acknowledging social and anthropological of the environment where the projects are done is necessary and mandatory before starting the project and will assist the related systems to develop projects. In order to implement projects, it better to use local facilities, equipment, and work force to the extent possible. This may on the one hand lead to employment and on the other hand a kind of compassion in keeping and preserving projects among local residents.

Supervision and investigation on correct performance and protecting from performed projects in the area of Sistan should be specified and clarified. like preparing conditions toward constant local development relying on local capitals and increasing the residents' role of the area in developing the area. The experts be allowed to participate voluntarily and with complete knowledge and they be avoided compulsory participation in performing the projects.

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Table 1: The Mann Whitney Test for the Indices under Investigation in the Projects under Study

average scores			Asymp. Sig .(2-tailed)	Mann-Whitney u	Indicators	Projects
Mean Rank	N	Group	0/649	1816/500	employment	water transfer from Sistan to Zahedan
61/87	56	administrative experts	0/717	1/835	income	
			0/311	1/709	immigration	
63/02	68	non-administrative experts	0/680	1/826	development	
			0/886	1843/000	hope for survival	
-	124	Total	0/625	1810/500	business thriving	
			0/852	1868/500	livelihood	
Mean Rank	N	Group	0/084	1569/500	employment	Drainage of Sistan
57/81	56	administrative experts	0/173	1/642	income	
			0/530	1/783	immigration	
66/36	68	non-administrative experts	0/202	1/659	development	
			0/056	1537/000	hope for survival	
-	124	Total	0/047	1525/000	business thriving	
			0/039	1506/500	livelihood	
Mean Rank	N	Group	0/508	1745/000	employment	fourth half well dam
60/09	56	administrative experts	0/511	1/746	income	
			0/831	1/830	immigration	
64/49	68	non-administrative	0/763	1/780	development	
			0/634	1780/500	hope for survival	

		experts				
-	124	Total	0/802	1823/000	business thriving	
			0/796	1821/500	livelihood	
Mean Rank	N	Group	0/394	1743/500	employment	project of Chabahar – Milak transit road
65/37	56	administrative experts	0/153	1/632	income	
			0/105	1/592	immigration	
60/14	68	non-administrative experts	0/933	1/888	development	
			0/667	1822/000	hope for survival	
-	124	Total	0/117	1606/000	business thriving	
			0/342	1724/000	livelihood	
Mean Rank	N	Group	0/012	1418/500	employment	cement factory
71/21	56	administrative experts	0/006	1/372	income	
			0/012	1/416	immigration	
55/33	68	non-administrative experts	0/060	1/541	development	
			0/019	1447/500	hope for survival	
-	124	Total	0/022	1459/000	business thriving	
			0/009	1347/500	livelihood	
Mean Rank	N	Group	0/674	1823/500	employment	changing regional space organization from 1 city to 3 cities
57/24	56	administrative experts	0/125	1/610	income	
			0/760	1/846	immigration	
66/83	68	non-administrative experts	0/511	1/778	development	
			0/211	1662/000	hope for survival	
-	124	Total	0/278	1695/000	business thriving	
			0/235	1675/500	livelihood	
Mean Rank	N	Group	0/557	1807/500	employment	developing higher education
63/62	56	administrative experts	0/405	1759/500	income	
			0/971	1913/000	immigration	

61/31	68	non-administrative experts	0/709	1848/500	development	
			0/833	1879/500	hope for survival	
-	124	Total	0/523	1797/000	business thriving	
			0/992	1918/000	livelihood	
Mean Rank	N	Group	0/557	1807/500	employment	changing settlement sites into towns
60/76	56	administrative experts	0/405	1759/500	income	
			0/971	1913/000	immigration	
63/93	68	non-administrative experts	0/709	1848/500	development	
			0/833	1879/500	hope for survival	
-	124	Total	0/523	1797/000	business thriving	
			0/992	1918/000	livelihood	
Mean Rank	N	Group	0/185	1653/500	employment	transit road
63/88	56	administrative experts	0/315	1/715	income	
			0/389	1/827	immigration	
61/37	68	non-administrative experts	0/498	1/774	development	
			0/457	1762/000	hope for survival	
-	124	Total	0/675	1824/000	business thriving	
			0/361	1730/500	livelihood	
Mean Rank	N	Group	0/290	1703/500	employment	border wall
66/55	56	administrative experts	0/228	1/677	income	
			0/649	1/818	immigration	
59/16	68	non-administrative experts	0/354	1/728	development	
			0/696	1830/000	hope for survival	
-	124	Total	0/478	1770/500	business thriving	
			0/941	1890/000	livelihood	

Source: research findings, 2014

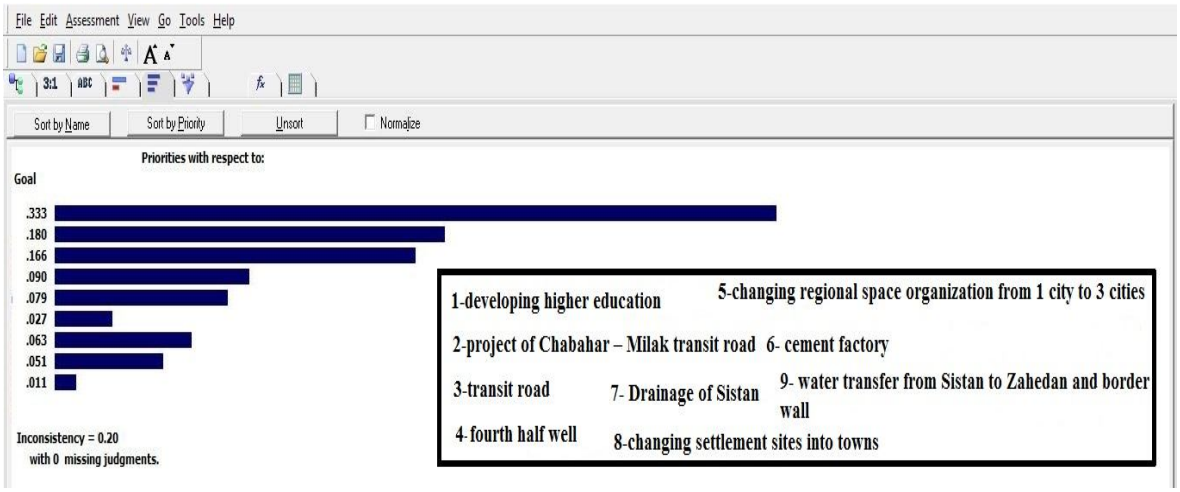

Figure 1: Weights obtained from overall paired comparisons of the projects under investigation

Table 2: Average Scores

	N	Mean Rank	Sum of Ranks
administrative experts	568	629.91	357788.00
non-administrative experts	672	612.55	41163200
Total	1240		

Source: research findings, 2014

Table 3: Mann-Whitney Employment Index

	employment
Mann-Whitney U	189012.500
Wilcoxon W	415140.500
Z	0.300-
Asymp. Sig. (2-tailed)	0.764

Source: research findings, 2014

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RESEARCH ARTICLE

Survey of Microbial Contamination of Cold Water Fish Marketed in the Fish Supply Centers in Urmia City, With an Emphasis on, Aeromonas Hydrophila Bacteria

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ABSTRACT

Rainbow trout is of farmed cold water fish that its supply performs in stores offering live aquatic products and packaging. The present study aimed to assess the microbial contamination of cold water fish offering in live fish supply centers of Urmia city, with emphasis on Aeromonas hydrophila bacteria. Studied bacteria, including pathogenic bacteria transmitted to humans that imported a lot of financial and physical losses to fish farming industry. In the present survey, 300 samples were taken from live fish supply stores and 5 organs, skin, liver, kidney, spleen and mouth were surveyed in terms of the presence of three bacteria studied. Sampling was conducted randomly. In the microbiological survey, the presence of the bacteria was proved in the organs studied. The most frequency of A. hydrophila bacteria was in skin and this bacterium was not isolated from liver, kidney and spleen organs. Presence of the bacteria in fish studied shows the contamination of state farms and necessitates the health care in these fields.

Key words: Aeromonas hydrophila, Urmia, Oncorhynchus mykiss

INTRODUCTION

Aeromonas hydrophila is a heterotrophic, Gram-negative, rod-shaped bacterium mainly found in areas with a warm climate. This bacterium can be found in fresh or brackish water. It can survive in aerobic and anaerobic environments, and can digest materials such as gelatin and hemoglobin. A. hydrophila was isolated from humans and animals in the 1950s. It is the most well known of the species of Aeromonas. It is resistant to most common antibiotics and cold temperatures.

Because of its structure, it is very toxic to many organisms. When it enters the body of its victim, it travels through the bloodstream to the first available organ. It produces aerolysin cytotoxic enterotoxin that can cause tissue damage. A. hydrophila, A. caviae, and A. sobria are all considered to be opportunistic pathogens, meaning they rarely infect healthy individuals. A. hydrophila is widely considered a major fish and amphibian pathogen (1), and its pathogenicity in humans has been recognized for decades (2). The genomic insights of aeromonads could be a stepping stone into understanding of them (3).

A. hydrophila is not as pathogenic to humans as it is to fish and amphibians. One of the diseases it can cause in humans, gastroenteritis, occurs mostly in young children and people who have compromised immune systems or growth problems. This bacterium is linked to two types of gastroenteritis. The first type is a disease similar to cholera, which causes rice-water diarrhea. The other type is dysenteric gastroenteritis, which causes loose stools filled with blood and mucus. Dysenteric gastroenteritis is the most severe out of the two types, and can last for several weeks. A. hydrophila is also associated with cellulitis. It also causes diseases such as myonecrosis and eczema in people with compromised or suppressed (by medication) immune systems. (4) In very rare cases, A. hydrophila can cause necrotizing fasciitis. (5)

MATERIALS AND METHODS

Study on rainbow trout that live in the center of the city of Urmia was sold. The purpose of this study was to obtain samples of the bacteria Aeromonas hydrophila. To do this, 300 samples were collected from different centers in groups of 10 teeth were moved along the ice.

To check data normality test Kolmogorov - Smirnov test and for homogeneity of variances levene for statistical analysis of data from two-way analysis of variance (ANOVA) was used. To evaluate the differences between the mean values obtained at different times for the two treatments in a time of LSD and Duncan test was used at 95%. For statistical analysis software SPSS version 18 was used to draw diagrams of software MedCalc v12,1.4.

To investigate the presence of bacteria, samples were collected in blood agar and nutrient agar medium in surface culture and the cultures were incubated for 24 h at 25 ° C. To purified samples, subculture in environments listed on separate plates were cultured and then incubated for 24 h at 25 ° C, respectively.

To investigate the presence of bacteria, samples were collected in blood agar and nutrient agar medium in surface culture and the cultures were incubated for 24 h at 25 ° C. To purify the samples mentioned in the subculture of the colonies with different morphologies on the plate isolated and cultured again for 24 hours at 25 ° were incubation. This is the stage where there is a colony of Gram staining was performed. After confirming acceptable purity for bacteria isolated and purified by subtraction and biochemical tests were conducted. The differentiation of lactose-free environments, indole, urea, gelatin and hemolysis tests, MR, VP, oxidase, catalase and Gram stain was used. In order to compare the positive and negative test cases in order to avoid possible false positive tests at every stage of differentiation, from a pure bacterial coding was used to confirm the integrity test (6). It should be noted that culture

and sampling procedures in compliance with aseptic conditions, with the flame, the hood has been done to prevent secondary pollution potential.

Venice subtraction results of all tests and biochemical tests using scientific source and microbiology reference books were interpreted (7).

RESULTS

Study to determine the presence of bacteria Aeromonas hydrophila in fish that live in the center of the city of Urmia is sold out. Then we will try to help the charts in the presence of micro-organisms including bacteria, pathogenic and transmissible to humans is to be dealt with.

According to data from the study of fish sold in Urmia city center offers live fish were contaminated with bacteria Aeromonas hydrophila. Various agencies of the bacterial contamination of samples varied. Most infections with the bacterium Aeromonas hydrophila bacteria from the skin and body organs, liver, kidney and spleen were isolated.

CONCLUSION

Manuselis, 2000 in the presence of Aeromonas hydrophila bacteria in drinking water and even different regions frequently reported et al. (8, 9). Tavakoli et al in 1387 demonstrated that the bacterium Aeromonas hydrophila strain of rainbow trout serum immunoglobulin also increased, decreased hematocrit and red blood cells. (10). By comparing the results of the research that has been done in the past by local and foreign researchers can say that this bacterium Aeromonas hydrophila in fish farms have been present. The presence of these bacteria from the two aspects of public health and the heavy casualties caused by the contamination of farms can be evaluated. This made the flaws in the health care field will cause the emission of water resources in the province. As well as patients and their causes damage to life and property.

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Table 1: Prevalence of infection with the bacterium Aeromonas hydrophila in terms of the absolute number of cases

Organs examined	Mouth	spleen	kidney	liver	skin
number Of pollution	2	0	0	0	82
percentage of pollution	7	0	0	0	27/3

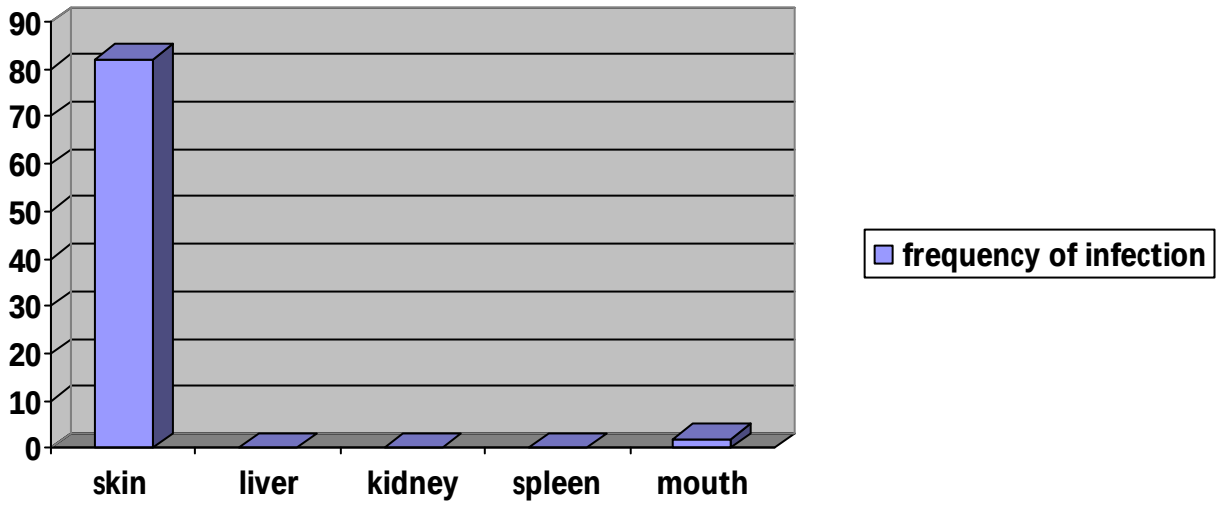

Figure 1: Absolute frequency of infection with the bacterium Aeromonas hydrophila by the members of the study.

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RESEARCH ARTICLE

Structure and the Roots of the Obstacles to the Belief in Resurrection with an Emphasis on the Psychological Approach

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ABSTRACT

The objective of the present study is to explain structure and roots of obstacles to the belief in resurrection with an emphasis on the psychological approach. In this study, the obstacles to the belief in resurrection have been examined in three areas that are: perceptual, attitudinal and behavioral. The purpose of the structure of the obstacles to the belief in resurrection with an emphasis on the psychological approach is to examine the role of dimensions and components of sensual realm, including cognitive, emotional and behavioral components in obstacles to the belief in resurrection and also to evaluate the quality of the relationship between them. In this research, "faith" and "belief" and "attitude" have been considered to have same meaning. Human has three "ideological, emotional and volitional" aspects. Three dimensions of (intellect, will, and emotions) are in mutualinteraction and influence with each other. This means that the cognitive dimension has a direct influence on emotional and volitional dimensions and the emotional and volitional dimensions affect the cognitive and epistemic dominion of human.The method used in this research is descriptive – analytical method.The data were collected through the libraries survey with reference to the original sources and also available software in the form scientific files by recording data in research files. Among the components of cognitive obstacles, "ignorance", is the most effective obstacles to belief in the resurrection. Therefore, the "ignorance" is one of the fundamental obstacles that have a crucial role in the emergence of other cognitive obstacles; this means that there is no cognitive obstacle in which the "ignorance" is not involved. "Whim", among the components of emotional obstacles, has an influential role in the lack of belief in the resurrection. "Whim", means "an excessive want or desire" or "unlawful and improper" desire. Therefore "whim" is one of the main and fundamental

obstacles that have an essential role in the emergence of other obstacles. Every sin and evil behavior, such as oppression, lie, suspicion and arrogance ..., cause disturbance in cognitive and emotional function of humans, in a way that in the case of insistence on sin, the human losses his insight and perspective. Thus sinful and deviant behaviors become the reason for forgetfulness and negligence of the resurrection and the belief in the life hereafter.

Key words: Obstacles, Resurrection, Structure, Psychology, Insight, Attitude, Action

INTRODUCTION

Resurrection is one of the principles and components of the religion of Islam, and has the greatest impact on the development of the human personality (mind, will, behavior). Existential dimensions of human consist of perceptual-cognitive, attitudinal -emotional, behavioral-factional dimensions; accordingly, in this studythe obstacles to the belief in resurrection have been examined in three perceptual, attitudinal and behavioral areas. Considering that the faith is an equivocal concept, we cannot divide the people into the two groups of those who believe in resurrection and those who deny the resurrection, because there are different grades and diversities regarding the belief of people in resurrection. “Gradation”, since belief in resurrection has intensity and weakness, that is, according to its degree, it is graded. "Diversity", since the dedication of individuals to Islamic teachings is not same and according to internal and external circumstances, they show greater commitment to some of them and neglect the other ones. The purpose of the structure of the obstacles to the belief in resurrection with a psychological approach is its dimensions and components (cognitive, emotional and behavioral)and thetype of the relationship between them. The purpose of this study is to answer the main question that: What is the structure of obstacles to the belief in resurrection, with a psychological approach? In other words, what are the obstacles to belief in resurrection according to the sensual dimensions of insight, attitude and action? Considering the fact that the life of human is a reflection of his thoughts and emotions and the unique and unmatched role of faith and denial of resurrection in individual and social life, study of this issue is of great importance; because in Islamic thought, denial of the resurrection is considered to be the root of all evils, violations and sins. Individual sins and familial, social and political conflicts are rooted in denial of the resurrection and the role of it cannot be denied, therefore, evaluation of this issue in a scientific- Qur’anicstudy is considered as a necessity. Despite numerous works and researches that have been conducted about the principle of "resurrection", the "obstacles to resurrection belief" have not been discussed directly. In the Qur’anic verses, as an important religious source, the objection and resistance of the ethnicities, such as Noah, Hud, Joseph, Moses, Lot, Sheba and… against the mission of the prophets have been mentioned that the common feature of all of them was denial of the Resurrection and the Day of Judgment.By searching the website of Iranian libraries (lib.ir) and digital libraries of Noor and specialized magazines of Noormags and… no book or dissertation and/or an article was found that have dealt with this subject specifically and independently Y, but some commentators in their commentary books in interpreting the verses relating to the denial of the resurrection, have spoken about the way of its formation and its effect on the human psyche. For example, the "pride" has been mentioned as one of the obstacles of the belief in resurrection, that in the verse 22 of Surah AN-NAHL (THE BEE) and in interpretations, it has been discussed:Al-Tibbyan Fi Tafsir al-Quran (Qur'anic Commentary Known as Tibyan), Majma‘al-Bayan fi-Tafsir al-Qur'an (Collection of discourse in the interpretation of the Qur'an), Mafatih al-Ghayb (Keys to the Unknown),Anwār al-TanzīlwaAsrār al-Ta'wīl(The radiances of revelation and the mysteries of exegesis),Ruh al-Bayan (the Spirit of Elucidation),Al-Mizan fi Tafsir al-Qur'an (The Balance in Interpretation of the Quran), Fi Zilal al-Quran (In the shade of the Quran), TafsirNemooneh (The Ideal Commentary)and….

In the new researches, an independent study concerning this field, in Persian, has been observed. New researches are predominantly based on empirical research and provide the origin of religion in general, like the works in the psychology of religion that have dealt with the general topics about the religion such as the origin of religion, the impact of religious teachings on human behavior and the relationship between religion and death: The psychology of

religion (Bernard Spilka and others), Psychology of religion from the perspective of William James (Azerbaijani), Psychology of religion (David Wolfe), Religion and psychology (William James). Also the doctoral dissertation entitled as "Study of psychological factors influencing religious belief from the perspective of Islamic teachings and explaining them from the point of view of psychology of religion" was conducted by Ali Hadian, with the guidance of Seyed Mohammad Gharavi in Qom University, Faculty Islamic sciences and Theology, in 2012. As the background of the research implies, the present article is a novel one of its type, that is, the study of obstacles to belief in resurrection (denial of resurrection) with a psychological approach and on the basis of the sensual aspects (insight, attitude and action) is something new and unprecedented.

Structure of obstacles to resurrection belief

Initially the roles of each of the existential dimensions of human, including cognitive, emotional and behavioral dimensions are discussed and then the relationships between the three dimensions are scientifically examined.

Perceptual- cognitive

Perceptual and cognitive obstacles are the first dimension in the structure of obstacles to resurrection belief. Due to the role and impact of the knowledge and awareness in the all stages of man's evolution and growth and since the human reaches a degree of faith and belief through accomplishing these steps and gains a power to identify wrong and right and the normal and abnormal, its role in the belief or disbelief in the resurrection (in case of deficiency of knowledge) is essential. In religious teachings, much emphasis has been placed on the acquisition of knowledge and the use of power of reason, and for this reason, in the Islamic texts, it has been recommended to acquire knowledge without any restrictions. “Seek knowledge from cradle to grave” (Gilani, 1998, 606, Tousi, 1390, 1, 11, FeizKashani, 1986, 1/126), “One hour of thinking is better than one year of worship” (Gilani former, 114, Majlesi, 1403, 71/325, 20), because the knowledge and awareness about the human needs is very impressive in the human life. In a narration from Imam Hassan (AS), he says: I wonder those who think about their body's food, but do not think about their soul's food. They keep away disturbing food from their belly, but fill up their heart with destructive subjects! (Majlesi, former, 1/218, H43; Alravandy, 1407, 144, H375) Hence, to make decisions to be rational, Imam Ali (AS) ordered that: “All the movements and actions require a realization and knowledge” (Harrani, 1404, p. 171). Appropriate and true knowledge and awareness towards the realities of the universe, warn and aware the human to answer the question that where he has come from? And what is the reason of his existence? And where he will go? And this is the origin for self-realization, which is the highest and most beneficial kind of knowledge (Majlesi, former, 2/32, H-22). Recognition of Almighty God and awareness about the fact that the creation of man and the universe is purposeful, as well as recognition of the resurrection forms the "universal consciousness" of the human that this awareness will affect all components of the human personality. In a narrative from Imam Sadiq (AS), he statesthat: “I have found all knowledge in four things; to get to know your Lord, to know what He has given to you, to know what He wants from you, and to know what takes you out of your religion "(Koleini, 1388, 1/50, H-11). By acquisitions of this knowledge and considering their surroundings, human beings acquire the fundamental knowledge and awareness that whatever of creatures be it big or small, valuable or worthless, have been created purposely (Majlesi, former, 3/136). The human, in proportion to his creation, moves towards perfection and growth and satisfaction of his needs, now, if despite the cognitive ability and the tools provided for the human being, he refuses to take a step in the field of self-knowledge, theology, cosmology and eschatology and be inattentive regarding the required knowledge and be negligent towards knowing and understanding of the facts such as resurrection, he will get trapped in obstacles such as ignorance, imitation, narrow-mindedness, doubts and wrong application of cognitive tool ... that these obstacles are considered as components of perceptual and cognitive obstacles.

Among the components of cognitive obstacles, "ignorance" is the most effective obstacle to belief in the resurrection. "Ignorance," is a equivocal concept that means "not knowing or lack of knowledge", against the concept of "science" of that means "knowing or knowledge", and in other words, to the extent that the knowledge is effective in the human faithin believing the resurrection, ignorance, in the opposite direction, causes the fall, misguidance and denial of the resurrection and in narrative texts, the ignorance has been mentioned as spoiler of resurrection (TamimiAmadi, 1366, H-848), most humiliated humiliation (VaramIbnAbiFiras, 1989, 70, H-65), the root of all ill deeds (TamimiAmadi, former H-819), the spoiler of the world and the Hereafter (FattalAlneysaburi, 1406 AH, 17) and the worst disease (TamimiAmadi, former H-2882); therefore, the "ignorance" is one of the fundamental obstacles that have a crucial role in the emergence of other cognitive obstacles, this means that there is no cognitive obstacle in which the "ignorance" is not involved.

Attitudinal - emotional

Attitudinal - emotional obstacle is the second dimension in the structure of obstacles to belief in resurrection. In the definition of emotion it has been stated that: "Emotion is a complex emotional state which is along with psychological, physical and behavioral components. Emotion has an outward manifestation which is called affect and an inner manifestation which is called mood. Others can participate in outward manifestation of emotion; but inner manifestation of emotion is inner and individual feeling which cannot be shared with others or communicated to them, for example, an inner mental joy, does not have any external manifestation (Majd, 2006, 109 and 113). Emotions and desires of human are among the important component of personality and a part of the mental world of human, in a way that no voluntary and optional work can be realized without desire and they have very important role in human life and at all stages of formation and growth of human, and individual and collective behavior of humans, are influenced by emotions, even mental, intellectual health and behavior of human are dependent to emotional health, and if the desires and emotions of the human are not managed and developed naturally and in a right and proper direction, the human would be inhibited from belief in facts such as faith in origin and the resurrection and due to causing an interest and attachment to material things and worldly affairs, the man will be restrained from his evolutionary growth and movement. In this section, we can mention three important obstacles of "secularism", pride (pride and arrogance) and following the whims. "Whim", among the components of emotional obstacles, has an influential role in the lack of belief in the resurrection. "Whim", means "an excessive want or desire" or "unlawful and improper" desire and that is why, it is blamed (Askari, Bita, 117), or it is the desire of the soul to lust (RaghebIsfahani, 1412, 849), and in narrative text, the whim has been referred to as the foundation of sufferings and misery (TamimiAmadi, former, H. 1048), the biggest enemy (Ibid, H-1678), The pest of wisdom and intellect(ibid, H. 3925), causative of blindness and deafness and humiliation and misconduct (Ibid, H-9168). Therefore "whim" is one of the fundamental and main obstacles that have an essential role in the emergence of other obstacles. The person who possesses this condemned attribute is expected to do any mischief and to satisfy his inappropriate and irrational desires he would not withhold any deception and trickery.

Functional -behavioral

Behavioral obstacle is the third dimension of the structure of obstacles to belief in resurrection. Behavior is the outcome of the other two dimensions, namely, "insight" and "attitude", and the purpose of functional obstacles is abnormal behaviors that by influencing the insights and attitude cause the human to neglect and forget the afterlife. In Islam, behavior and actions, which are out of the religious framework and principles, are considered "sin" or "unlawful". In other words, sin is any behavior that is contrary to God's command (IbnManzur, 1414, 1/389, Farahidi, 1410, 8/190). In religious texts, sin or abnormal behavior has been divided to superficial or outward sins (oppression, backbiting, lying, etc.) and inward or intrinsic sins (idolatry, disbelief, suspicion, pride and arrogance, etc.)

Verses and narrations emphasize on the fact that the reprehensible and malicious actions and behavior will damage the mental health and safety: "no indeed! Their own deeds have cast a veil over their hearts [who consider the facts to be fable]. (AL-MUTAFFIFIN (DEFRAUDING)/14) Inappropriate and sinful behavior is the reason of anxiety and illness that threatens the mental health of human (Koleini, 1388, 2/275, H-28), and nothing can corrupt the heart like the wrong and sinful behaviors (Ibid, 2/268, H-1). All sins, whether trivial or great, small or much, has negative effects on the safe and healthy psyche and the repetition of sins makes it to be a normal thing for the human, in a way that he becomes unaware about the sinful aspect of it and becomes as the part of his nature and temperament. The common feature of these sinful and deviant behaviors is violation of divine commands and continuity of sin (AL-QALAM (THE PEN) / 12, AL-MUTAFFIFIN (DEFRAUDING)/12). Among the components of behavioral obstacles to resurrection belief, every sin and evil behavior, such as oppression, lie, suspicion and arrogance ..., that causes disturbance in cognitive and emotional function of human, in a way that he is not able to recognize the truth (AL-TAWBA(REPENTANCE) / 87), and the human heart losses its intuition and insights (YUNUS (JONAH) / 74) and becomes brutal (AL-BAQARA (THE COW) / 74), in that inverted heart there will be no goodness and compassion. Therefore sinful and deviant behaviors cause forgetfulness and negligence of the resurrection and the belief in eternal life.

The relationship between the existential dimensions and dominions of human

Three dimensions of (intellect, will, and emotions) are in mutual interaction and influence with each other. This means that the cognitive dimension has a direct influence on emotional and volitional dimensions and the emotional and volitional dimensions affect the cognitive and epistemic dominion of human, hence, some believe that the voluntary behaviors of human in everyday life are derived from his "will" and no action and work van be performed until the human will is stimulated (Sheikh Eshragh, 1996, 4/208); Tousi, 1996, 2/423); and the will of human is also affected by his passion and emotion, and without having a desire and willingness for performing a task, the human will would not be motivated and passion and emotional aspects of human are also affected by cognitive dimension and his beliefs, thus when a person does not have a cognitive assessment about an action and does not know the positive and beneficial aspects of it, he won't show any enthusiasm and interest for performing that work, and consequently his will is not stimulated and the work is not performed (Tabatabai, Bita, Nihayat al-Hikmah, 121 and 122).Ghazali says: "Having knowledge about the goodness and good deeds is necessary, but it is not sufficient for operating them, and the human must possess the necessary will and emotions are the key and essential drivers of human behavior, when the stimulants and drivers are not raised, they would be in stealth state and for their actualization, they should be linked to desire and willingness" (Ghazali, 1985, 1/304, and 2/28, and 4/109), Mian Mohammad, 1986, 2/60). Some philosophers emphasize on the fact that human beings have the will and intellect and his works and behaviors are caused by a sense of obligation and moral responsibility, but undoubtedly inner forces of human (feelings and emotions), have great effect on human thought and behavior, such a way that they encourage him to take some positions and also inhibit him from taking the other position, or directs him towards a kind of thinking and prohibits him from the other kind (Tabatabai, Bita, former). Considering that the feelings and emotions are an integral part of the human characters and also given their effect on human thought and behavior, Islam in its moral concepts and educational approaches, has placed a great emphasize on the psychological attitudes and reactions (such as friendship, enmity, anger, happiness, fear and hope ...) (Moen, 2008, 152). Thus, man is a creature, who is mixed of intellect and emotion, love and thoughts, and each of these dimensions play a major role in mobilizing and exalting the human and we can never accept one of these dimensions and ignore the other, neither the mind can do the work of heart nor the heart can do the work of mind.

The effect of emotion on the thought

The effect of emotion on the thought, whether positive or negative, is essential, because sometimes certain emotions become strong to the extent that prevents man from thinking. Prophet of Islam (PBUH) said: "Your love and interest towards anything, makes you blind and deaf." (Sadough, 1404, 4/380) It means that whenever you love something,

you cannot see its defects and you cannot hear the words of those who criticize it, so in this way you become blind and deaf about the thing you love and you can no longer see and even hear its negative points and weaknesses, because the spirit which dominates such friendships is not wisely and rationally, but rather the source of the start and continuation of such relationships, is physical lust and bestial pleasures, in this kind of loves, the attention of lover is only to the appearance of beloved and sees nothing but the face, shape and color. Imam Ali (AS) said: "The eyes of the lover are blind to see the disadvantages of the beloved and his ears are deaf to hear the obscenities of the beloved "(TamimiAmadi, 1987, 481). Emotions are the most important element of the personality of human that effect the decisions of human, because human thinks through his emotion and looks at things via the emotional perspective, and through it, prefers some external or internal stimulus over the others, that is why human's decisions and opinions are influenced by the love of the homeland and religion, which is called "logic of emotions" (Osman, 1387, 2, 172). Also Imam Ali (AS) stated that: "He, who loves something, has blinded his eyes and has sickened his heart"(Razi, Bita, 109, 160), it means that,the lover sees the things incorrectly and hears the things with deaf ears,lusts disables him to think correctly and the world, takes his heart, and his soul wants it unquestionably. Worldly and secular man, is the servant of the world and those people who have something of world in their possession and he will follow the other people, he will follow their path and advocate whatever they advocate he will advocate too and no inhibitors can prevent him from doing evil and he will not accept any advice from any adviser.Imam Sadiq (AS) said "thecommander of the faithful, Ali (PBUH)in preaching to one of his companions wrote: "Desist the world, that the friendship of the world makes people blind and deaf and dumb and bends the necks with humiliation "(Majlesi, former, 70, 75). Imam Ali (AS) said: "mind is not accumulated with lust" (TamimiAmadi, former, 65).All types of passivity, especially extreme passivityare not compatible with healthy thinking and good manners,therefore the most wise people are those who have less anger (extreme and irrational), because the anger, is the nightmare of human mind (Ghazali, former, 3, 163)and when the intellect becomes weak, the devil plays with it as a child plays with the ball,wrath and anger paralyzes the ability of correct judgment (Osman, 1387, 149 and 150).According to Hume,ideas form our emotional feelings and in a reversal, beliefs, come into being by emotions;therefore, emotions are not identified solely by means of one feeling,but rather they are detected by a complex web of emotions and ideas.Hume's emphasis on the emotional status of thought, is today known as the "cognitive domain of emotions" (ibid, 72).Hume believes that through reason, no desire or resentment will be created in human, desires and resentments are from the category of emotions and passiveness and emotions, or passivity, are the powers which are other that the power of "reason",although in the philosophy and even in ordinary life, usually in the conflict between the passivity and wisdom, the wisdom is preferred and they insist on the fact that the humans are virtuous to the extent that they submit themselves to the commands of their reason,but I will try to prove that reason, alone, can never be a motive for our voluntary actions."Emotion or passivity are encouraging and provocative, but the reason is quite bare and ineffective" (Trick, 2003, 112).Therefore it is during a pain or pleasures that tendency or aversion toward an object rises,and subsequently, we feel emotions, tendencies or resentment,and we are persuaded toapproach or avoid what provides happiness or unhappiness for us (Kalbali (Fayaz), 2006, 75).So fundamental motive of human actions is not the reason, but rather inclination and evasion that are caused by experiences of pleasure and pain,so reason is not the onlyefficient cause of behaviors, but it is considered as a passive tool (Trick, 2003, 112).According to him,"reason and feeling are in cooperation in almost all moral decisions and conclusions, but the final decision that makes the actions pleasant and the laudable or vexatious and blaming,is the "sense or inner feeling" that the nature has made it inclusive in all mankind.He believes that the reason can prevent the will when it creates a desire and motivationin the direction opposite to the direction of passivity, so that this desire and motivation can enable other wills, because nothing can fight with the passive motivation, unless the opposite incentive.Hume says that "wisdom is captured by emotions", because the reason cannot regulate the ends and the goals for us andit just shows that how we can obtain what we have wanted previously (Ibid).

The effect of emotion on behavior

In the domain of behavior, the emotions act like an incentive and stimulus,because life without emotion and excitementresembles to silence and death;Thus, the role of emotion and excitement in the efforts and attempts of human to defend himself and to survive the life and to create appropriate contexts in different situations, is essential.One of the important functions of the excitement is a rewarding and punishing behavior, it means that, when a person experiences very positive emotion, he would repeat the same behaviors that cause the same emotion and excitement, similarly, when he experiences negative emotions, he would avoid the behaviors that cause the same emotion (Franken, 2009, 408). In Holy Quran, it is stated that: “say (ProphetMuhammad): 'if you love Allah, follow me and Allah will love you” (AL-E-IMRAN (THE FAMILY OF 'IMRAN)/31)

Therefore, anyone who has faithful love and hatred, always remembers the beloved, whether he is at work,leisure, study and ...at all the times he remembers the beloved.Dear Prophet (PBUH) said: the sign of loving God is to love remembrance of Him (Majlesi, the former, 69, 252, H-8),and remembrance of God, means obedience from the redemptive commandments of God and theluminous statements of innocent saints.God, Mighty and Exalted, said to Moses, "O son of 'Imran, I am aware of my friends. They become different at night, as if they have been uprooted from their place. Requital almost comes before their eyes. Then they address me as if they see me and they talk to me from the near(Majlesi, former, 13/329).Therefore, those who, night and the day, talk about their love for God and the Infallible Imams (AS) and the virtuous and righteous people, but they act completely different from them and are guilty and sinful, according to the logic of Islam are totally alien and nothing but a liar; therefore, love or affection is a practical interest and God puts His mercy upon the one who loves God and obeys his commands. Imam Baqer (AS) said: “No one is our Shia except by the fear of God and His obedience, and they are not recognized except by their humbleness, reverence, trustworthiness, and abundance of remembrance of God, fasting, praying, doing good to parents, caring about poor and indebtedneighbors, caring aboutthe orphans, truthfulness, recitation of Quran, praising others and being trustee and reliable”(Harani, former, 215).Ghazali believes that there exists many emotions in the humans (Ghazali, former, 4, 366),that these emotions and desires, are the drivers of human behavior. According to him,supreme desires of man are the desires that make man to obey God and have a competent and righteous life that these desires act under the control of human and under the supervision of his wisdom and contrivance (ibid, 109).

CONCLUSION

In short, the human personality reflects his insight (knowledge), attitudes (emotion) and action (behavior).Improper beliefs and negative emotions and misconducts have an important role in denying the facts, especially the denial of the resurrection.The origin of many of the positive and negative emotions and also the good and evil behaviors of humanshould be sought in the type of his beliefs. Among the components of cognitive obstacles, "ignorance" is the most effective obstacles to belief in the resurrection. In other words, to the extent that the knowledge is effective in the faith and belief in resurrection, ignorance, in opposite direction, causes the fall and misguidance and denial of the resurrection.Therefore, the "ignorance" is one of the fundamental obstacles that have a crucial role in the emergence of other cognitive obstacles; this means that there is no cognitive obstacle in which the "ignorance" is not involved.Feelings and emotions are also considered as one of the important components of the human personality and they are an integral part of the mental world of the human and play an important role in his life, and the human life, at all stages of development, is influenced by his emotions, because human thinks through his emotion and looks at everything emotionally."Whim", among the components of emotional obstacles, has an influential role in the lack of belief in the resurrection. "Whim", means "an excessive want or desire” or "unlawful and improper" desire. Therefore "whim" is one of the main and fundamental obstacles that have an essential role in the emergence of other obstacles.In expression of behavioral obstacles to resurrection belief, it should be said that: every sin and evil behavior, such as oppression, lie, suspicion and arrogance ..., cause disturbance in cognitive and emotional function

of humans, in a way that in the case of insistence on sin, the human losses his insight and perspective.Thus sinful and deviant behaviors become the reason for forgetfulness and negligence of the resurrection and the belief in the life hereafter.

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RESEARCH ARTICLE

Studying the Efficacy of Cognitive-Behavioural Therapy to Reduce Loneliness and Depression in Children

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ABSTRACT

This study aimed to assess the efficacy of cognitive-behavioral therapy to reduce loneliness and depression in children, based on the relationship between mother and child. This study was a trial research with experimental and control groups. The research/survey consisted of all mothers with children aged between 7 to 12 years old who visit/ came to the centre for psychological counselling and services in the city of Sirjan, during the first 6 months of 2014 and whose children were eligible for/qualified as being depressed and lonely according to depression and loneliness test. Of these mothers, 30 were randomly selected and were placed in two groups of experimental and control groups. The intervention consisted of 10 sessions of cognitive-behavioral training program based on the relationship between mother and child which was conducted on the trial group. At the end of the course, a post-test was carried out on children in both groups. The results of covariance analysis showed that cognitive-behavioral therapy based on the relationship between mother and child significantly reduced the depression and loneliness among children in the experimental group.

Key words: Cognitive-Behavioural Therapy Based on the Relationship between Mother and Child, Loneliness, Depression

INTRODUCTION

Loneliness is an unpleasant condition which is caused by the difference in person's desired interpersonal relationship and the actual relationship with others in real terms. This feeling is important in the since it relates to individual

emotional feelings as well as being related to social poverty, behavior and health in adults, adolescents and children. Despite the widespread experience of loneliness among people of all ages, loneliness in psychology is of history short (Qualter et al., 2013). There has been several definitions of loneliness. Asher and Pocket describe loneliness as people's cognitive awareness of their weakness in social and personal relations which results in the feelings of sadness, emptiness, or regret. Dunn & Bidoza (2007) have defined loneliness as feeling of isolation. In their view, loneliness in children indicates a failure and weakness in inter-personal relations with their peers that results in dissatisfaction of social relationships with other children. In studying the definitions provided by the experts regarding loneliness, there are 3 components identifiable. The first component is the social component of loneliness. Loneliness clearly reflects the failure to have a satisfied social relationship. However it cannot be considered to be the same as isolation, in other words there is a distinction between loneliness and isolationism. Trying to separate loneliness from isolationism leads to the second component. Loneliness is a subjective feeling that might not match with the external reality (Heinrich & Galon, 2006).

The third component consists of emotional aspects of loneliness. In most definitions provided, it has been mention of unpleasant feelings and the negative emotions which most people try to avoid. However, instead of considering loneliness as a negative feeling, some researchers associate it with anxiety, depression, the feeling of not being liked and such (Wright, 2005). Loneliness is a passage for the influx of other negative feelings that most people try to consciously or subconsciously avoid. People, who feel lonely, have low self-esteem, especially have doubts about their abilities and in their interpersonal behaviours they show shyness, embarrassment, anxiety, and lack of assertiveness, low risk taking and introversion (Dunn & Bidoza, 2007). Researches show that there are a lot of emotional and psychological problems in children and adolescents with loneliness. Problems such as being under pressure, anxiety, high risk behaviours (such as smoking, drug use, truancy from school, depression & alcoholism), shyness, low self-assertiveness and self-confidence (Vanhalst et al., 2012). Studies show that people with loneliness have a different pattern of social response than other people and feel more threaten in social confrontations. Studies also shows that loneliness at childhood can cause low emotional health in adolescence, as well as loneliness being highly related to depression in children. (Schinka et al., 2012). Depressed children often show bout and numbness and isolate themselves in a corner. Their inhibition of movement shows in the forms of having problems playing or doing any task or work that has been once enjoyable, while the feeling of not being liked is obvious in them. Depressive disorders in children have negative effects on normal personal & family performance and will continue into adulthood if not treated (Zachrisson, 2009). In the etiology of childhood depressive disorder, it has been mentioned of family history, environmental factors, social factors and parenting technique. Research literature in this area, indicates the importance of early child development environment, especially family environment. These researches emphasize of important roles parents, parenting styles and attachment between parent and children have and consider them to be affecting factors on developing, increasing or decreasing children problems (Jakobsen et al., 2012). The results of the research by Morris & Partners (2000) showed that there is a connection between harsh parenting and discipline such as physical punishment with aggression and behavior problems in children. Researches advised on creating a warm and friendly environment within family and loving relationship between parents and children to reduce behavioural problems in children. According to the deeds theory, emotional attachment is an important factor in ensuring social and emotional development of children in later years of their life. Attachment affects social development in children, in a sense that due to this attachment they accept the values and standards of the people who care for them. Studies have shown that high level of insecure attachment significantly relates to higher level of present depressive symptoms. They have also shown the significant affect attachment styles have on total score of depression and anxiety disorders (Zachrisson, 2009). Depression in children is one on the most challenging clinical and mental health subjects. Considering the death due to depression, they believe that a review of related records is necessary in order to achieve treatment options. In this regard, although primary prevention is always more efficient and effective, but the evidence suggests that secondary prevention (treatment interventions) also significantly reduces future psychosocial problems.

Among the approaches used, those such as drug therapy, play therapy, mud therapy and many other methods used to intervene in children's emotional problems and often their performance was put to the test. However, there is still a need for better therapies. While research suggests that treatment approaches based on the relationship between parent and child, he can have a better performance. However, there is still a need for better therapies. While research suggests that treatment approaches based on the relationship between parent and child, he can have a better performance. Group cognitive therapy based on parent-child relationships is among the treatments which has attracted the attention of many researchers and scholars during the last few years. In this therapy, parents, as a therapeutic agent, during treatment sessions learn how to create a warm and friendly environment at home and establish a non-judgmental and unconditional relationship with their child and in this way creates a sense of safety in them. Applying these skills by parents results in increasing a source of internal control in child (Landreth & Braton, 2006). One particular aspect of this is that instead of focusing on changing the child's behavior, change child perceptions of parents and the relationship between parent - child. In this approach, the behavior observed as a function of perception. Thus, a change in perception will be a change in behaviour (Foley & et al. 2000).

Glazer & partners (2000) performed a research using the child-parent relationships based therapy in a group of children between the ages of four to eight years olds. At the end of treatment, the parents in the trial group were able to identify measure and assess anxiety levels in their children and showed more acceptance and empathy toward them. Landreth & Kidron also showed that this method not only reduces stress in parents but also results in empathic engagement between parents and children which follows more acceptance from them. Thus, considering these research findings regarding connection between loneliness and depression in children with type of interactions with parents and the influence of the style of parenting, attachment parenting on depression in children, also taking into account the effect of therapy based on interaction between parent and child on improving the parents empathic responses and increasing their acceptance, the question is whether cognitive-behavioral therapy (CBT) based on the relationship between mother and child could decrease the loneliness and depression in children?

MATERIALS AND METHODS

The research group consisted of all mothers with children with depression, aged between 7 to 12 years old who had referred to the centre for psychological counselling and services in the city of Sirjan during the first 6 months of the year 2014. Of these mothers, 30 of them whose children were suffering from depression and loneliness were randomly and after explaining and completing the consent form, 15 of them were put in experimental group and 15 were put in control group. Experimental group were trained children for 10 sessions of 45 minute long under cognitive-behavioral based on relationship between mother and child whilst the control group didn't receive any training. At the end of the training course measuring for depression and loneliness carried out on both groups of children.

Measuring Tools

Loneliness measuring: (LQ): Asher and Wheeler's questionnaire (1998) was used to measure loneliness in children and adolescence. It has 22 questions to answers from the scale of 1 to 5(strongly disagree to strongly agree). Asher and Weeler showed that loneliness measurement, distinguish between abandoned children and those of other classes and groups (neglected, controversial and popular) and reported the reliability with Cronbach's alpha equal to 0/9. Psychometric criteria of this questionnaire have been evaluated by Azad Farsani (2013) Cronbach's alpha reported to be equal to 0.75.

Child's depression questionnaire (CDI): This is a self-assessment tool for depression in children between the ages of 7 to 17 years old which was developed by Quaks (1981) and consisted of 5 subscales of negative mood, interpersonal problems, inefficiency, lack of pleasure and negative self-esteem, with 27 triple sentence questions. The questions are scored from zero to 2. Zero score indicates the absence of symptoms, a score of 1 represents the average and a score of 2 indicates there is a clear sign, so the scores range from zero to 54, with higher scores indicating more depression. The total score is the sum of the scores on the subscales. Shakeh Nia and colleagues have reported Cronbach's alpha of 0.85.

RESULTS

In table 2, the results of analysis of multivariate covariance to compare post- tests of depression in two group by controlling effects of pre-test has been shown. According to the information provided in the table, it can be said that there is significant difference in at least one of the assessed variables between the two groups. For a closer look at the differences observed, the assessed variables between groups are examined.

Based on the results presented in the table of comparisons between groups, it can be said that there is a significant difference between control group and experimental group in terms of depression and subscales. According to the amounts presented in the table, it can be said that in spite of controlling the effects of pre-tests, there is a significant difference between two groups in terms of loneliness.

CONCLUSION

This study aimed to investigate the effectiveness of cognitive behavioral therapy based on the relationship between mother and child, on reducing depression and loneliness in children. Based on the results of the present study, there is statistically significant difference between average control group with average experimental group in terms of depression and loneliness and the use of cognitive behavioral therapy based on the relationship between mother and child has significantly reduce depression in the experimental group compared to control group. Although no studies found regarding effectiveness of this treatment on loneliness, this study in line with previous studies, is compatible with the findings of Glazer & et al. (2000), Kidron and Landreth (2010), Rajabpour (2011) and Shakenia (2013). The results from research by Rajabpour (2011) suggest that the group therapy of mother-child relationship have significant effects on reducing verbal aggression in preschool children. To explain this, it can be said that unsuitable family context and environment as well as unprincipled patenting can results to development and increasing depression and loneliness in children. Therefore it seems natural that by changing and improving parenting styles family context and how members interact with each other and especially improving the interaction of parents with children, behavioural and emotional problems will reduce. In general, the findings can be interpreted such that the parent-child group therapy can have a long lasting effect on reducing loneliness and depression in children and can result in relatively stable improvement in emotional problems in children. The effectiveness of the treatment period indicates continuing improvement of inter-family relationship and changing the style of the relationship between parent and child, which according to the research findings is the underlying cause of aggression in children. The findings of this study significantly indicates that the skills provided during therapy sessions such as reflective listening, therapeutic restriction, identifying and responding to child's feelings and giving them choice, will teach them to express their emotions in an acceptable way and to develop self-control in themselves. By focusing on improving child-parent relationships in this approach, it leads to child's confidence and sense of security and give them a chance to explore their environment and to establish relations with other. On the other hand, secure relationships with parent, increases child's emotional control and problem solving skills and control of emotions, problem solving and increases the compatible coping strategies in child which in itself leads to greater compatibility with the surrounding environment. Considering that this study was conducted on children with emotional problems in the city of Sirjan, it cannot be generalized to children from other cultures and age groups. In addition, caution

should be taken when generalizing the results of this study to other childhood disorders. Finally, this study doesn't have a follow-up phase and extending it to periods of time intervals needs caution.

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Table 1: **Average and standard deviation of the assessed variables in the pre-test and post-test according the groups**

		Pre-test		Post-test	
groups	**variable**	**Average**	**Standard deviation**	**average**	**Standard Deviation**
experimental	Interpersonal problems	7.53	3.8	3.86	0.831
	Inefficiency	6.73	2.76	3.2	1.37
	Anhedonia	10.06	3.23	5.53	1.35
	Negative mode	7.73	2.78	3.86	0.99
	Negative Self-esteem	8.2	1.61	4.8	1.37
	Depression	40.26	5.59	21.26	2.63
	loneliness	69.01	6.86	50.8	6.73
control	Interpersonal problems	8.4	3.2	7.66	3.86
	Inefficiency	6.66	3.13	6.93	2.73
	Anhedonia	9.6	3.71	9.86	3.33
	Negative mode	9	2.47	9.2	3.54
	Negative Self-esteem	7.8	2.51	8.2	1.61
	Depression	41.46	7.57	41.86	6.23
	Loneliness	68.26	6.93	69.4	8.04

Table 2: **Results of analysis of multivariate covariance to compare post- tests of depression in two group by controlling pre-test**.

	Value	**F**	**Assumed Degree of freedom**	**wrong Degree of freedom**	**Significance level**
Pillai effect	0.916	41.34	5	19	0.01
Wilks Lambda	0.084	41.34	5	19	0.01
Hotelling effect	10.88	41.34	5	19	0.01

Table 3: Comparisons between groups to examine the differences observed in the analysis of multivariate covariance

	Total squares	Degree of freedom	Average squares	F	Significance level
Interpersonal problems	106.24	1	106.24	92.42	0.01
Inefficiency	86.18	1	86.18	15.84	0.01
Anhedonia	152.67	1	152.67	21.77	0.01
Negative mode	161.64	1	161.64	2196	0.01
Negative Self-esteem	76.47	1	76.47	33.45	0.01
Depression	2852.3	1	2852.3	143.06	0.01

Table 4. Results of analysis of covariance to compare post- tests of loneliness in two group by controlling the pre-test.

	Total squares	Degree of freedom	Average squares	F	Significance level
Pre-test	15.47	1	15.47	0.27	0.6
Group	2564.94	1	2564.94	45.42	0.01
error	1524.53	27	56.46		

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RESEARCH ARTICLE

The Effectiveness of Narrative Therapy on the Creativity and Social Development of Children; (Institute for the Intellectual Development of Children and Young Adults of Sirjan)

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ABSTRACT

Narrative therapy is one of the good practices in the process of counseling and psychotherapy of children. The purpose of the present study is to examine the effectiveness of narrative therapy on the creativity and social development of children, which is conducted in the form of a research project in the pretest-posttest control group design. The statistical population includes all 6 to 12 years old children of Sirjan's Institute for the Intellectual Development of Children and Young Adults that by using the convenient sampling method 100 children were selected and the questionnaires were distributed among them and after the examination of questionnaires, by using the convenient sampling method, 30 children who had obtained low scores on the questionnaires were selected and divided into the two experimental and control groups, each with 15 subjects and then the therapy package was implemented on them. Vineland Social Maturity Scale (VSNS) and Abedi Creativity Test Questionnaire (ATC) were used as the data collection tools. Data were analyzed by using SPSS16 software and analysis of covariance. Research findings indicated that narrative therapy have had a significant effect in increasing the creativity of children. Also the present study revealed that narrative therapy has a dramatic effect on social development of children.

Key words: Narrative Therapy, Creativity, Social Development, Children

INTRODUCTION

Human is a social being and it is almost impossible for him to continue his life individually, human beings communicate with others once they are born and receive the first feelings (Song, 2009). Appropriate social behavior is learned only through training and it becomes a habit by rehearsing, the success in this process, more than anything, depends on the individual effort and desire, however, factors such as parents, family, teachers, friends, even the stuff that we read and the movies we see are also influential (Moghtaderi, 2011). On the other hand, it is the most important and the most basic human ability and the most fundamental factor of value creation that plays an absolutely crucial role in all aspects of life. Creativity and innovation are the highest gifts of God to man and the reason and factor of being the noblest of all creatures and God's Caliph. All sciences, technologies, industry, innovations, inventions, arts, literature, architecture, and in general, the basis of various civilizations from the beginning up to present time and all human achievements are diverse expressions of creativity and innovation. Human civilization and his life cannot be possible without creativity. That is why the issue of creativity and innovation are becoming important and necessary day by day and always get more attention, in a way that, today, the field of scientific studies and researches about the various aspects of creativity, has turned into very important and broad specialized scientific field with an interdisciplinary nature (Golestan Hashemi, 2008). To foster the creativity, we should do the things that, on the one hand, enhance our knowledge and experience as the raw material of creativity and on the other hand, modify and guide our way of thinking and improve our imagination and provide the grounds for ideation. We know that creativity is intellectual and mental activity to achieve novel ideas. So, the things that can stimulate the mind in this regard and provide us the opportunity to think and crystallize it in the outside world increase the creativity (Golestan Hashemi, 2010). In the current situation, creativity is not a necessity, but a requirement for the survival. Therefore, educational systems place a great emphasis on the education and training of people who have the ability to creatively solve unpredictable problems (Golestan Hashemi, 2004).Books are one of those factors that affect the social development and creativity. Book has a strong influence on the personality and worldview of a child and in fact makes his dreams and wishes. Book is a powerful tool in conveying the concepts, information and, ultimately, in development of the child's personality. Through the book, the child sees the face of society and becomes familiar with the roles and procedures that he should adopt. Saadi's ingenious emphasis on the interactions, self-assertion, the role of social skills in promoting personal and social goals and even the management of home and community, all indicate the proximity of literature with psychological issues and it plays an important role in enhancing social development and mental health (Bidgoli, 2003). Through the stories and folktales, children become acquainted with the facts and experiences of life. Impressionability from the characters in the story, improving the ability of understanding and expression, fostering creativity, language training, and increasing vocabulary of children are considered as the other educational and training effects of storytelling (Yousefi Louyeh, 2006). In fact, the story is a broad creativity by which anyone can retrieve apparently irrational ideas and replace them and propose the creative use of them. That's why the narrative approaches emphasis on the psychotherapy and stories play a significant role in the way of children's attitudes and realities (Yousefi Louyeh, 2008) Stories create the places for the children that are unexpected and surprising and yet possible and positive. The efforts of researchers and therapists have led to the use of literature and story and narrative therapy (Sanaat Negar, 2012). Several studies have been conducted regarding the efficiency and effectiveness of narrative therapy in psychological problems of children that among them we can refer to following studies: The use of narrative therapy to reduce anxiety and increase the feelings of mastery and competence and hope in children (Hanney L. and Kozlowska K. 2002), Treatment of conduct disorder (Arad 2004), Narrative therapy and children with hearing impairments (Furlonger, B.F. 1999), studies of Freedberg (1994), for the treatment of the two anxieties of separation and fear, Webb and Berrt (1995) in the study of care for children with emotional or behavioral disorders and Herman (1997) in helping children who have been sexually abused. Finally, Montero K. & Renee (2002) have also concluded that the techniques of narrative therapy and bibliotherapy have had a huge impact in the modification of children's actions and in persuading them to do the right things. Yousefi Louyeh et al. (2008), in their study on the impact of narrative therapy in decreasing the symptoms of anxiety disorder and comorbid disorders in children (10 -9 years old), showed that the symptoms of anxiety disorders and comorbid disorders had a significant reduction in all

subjects after the narrative therapy. Moghtaderi et al (2011), by clarifying the effectiveness of the instruction of life values in social development of preschool children and self-efficacy of their parents, showed that the difference between the two experimental and control groups have reached a significant level in terms of social development of children and self-efficacy of their parents. Thus, with regard to the mentioned subjects, the present study was conducted with the aim of determining the effectiveness of narrative therapy in enhancing the creativity and social development of children and by considering the hypothesis that narrative therapy is effective in enhancing the creativity and social development of children.

RESEARCH METHODS

In the present study, according to practical purposes and on the basis of data collection, quasi-experimental, pre-test and post-test research method was used. The statistical population includes all 6 to 12 years old children of Sirjan's Institute for the Intellectual Development of Children and Young Adults that by using the convenient sampling method 100 children were selected and the questionnaires were distributed among them and after the examination of questionnaires, by using the convenient sampling method, 30 children who had obtained low scores on the questionnaires were selected and divided into the two experimental and control groups, each with 15 subjects and then the therapy package was implemented on them. Field method was used for data collection and the tools that were used include:

Abedi Creativity Questionnaire

This questionnaire was developed by Abedi on the basis of the Torrance Creativity Questionnaire and has 60 questions for the assessment of the creativity that evaluates the 4 components of fluency, elaboration, originality and flexibility. Abedi implemented it in 1984 among 650 students and the reliability coefficient obtained from the originality, fluency, flexibility and elaboration was respectively 0.85, 84.82, 0.0 and 0.80.

Vineland Social Maturity Scale (VSNS)

Vineland Social Maturity Scale (VSNS) was developed by Edgar Doll in 1935 and it was revised in 1965. This test was developed according to the Stanford – Binet test and the difficulty level of 50%. Dr. Okhovat and Dr. Baraheni have adapted the Vineland test from its American model and have made some changes in its questions, commensurate with culture of Iran. Vineland Social Maturity Scale is one of the transformational scales, which is associated with the level of persons' ability in meeting their practical needs and accepting responsibility. Although this scale includes the age range from birth to more than 25 years old and up to twelve years old, it has distinct question for each age level, but after the age of twelve years old, between 12 and 15 years, 15 to 18, 18 to 20, 20 to 25 years and above 25 years, the questions are common. This scale is based upon assessing the social competence and abilities of the person in his daily life. Vineland maturity scale is divided into 8 categories which are: self-help general (SHG) (younger than 8 years old) like keeping oneself away from simple risks, self-help eating (SHE) (younger than 10 years), when the child can feed himself, self-help dressing (SHD) when the child can dress himself, self-direction (SD) like choosing clothes on his taste, occupation (O) like doing some household chores with a sense of responsibility, communication (C) such as communicating with others through writing letters, locomotion (L) like traveling to places that are distant from homes and the last is socialization (S) such as participation in group activities. The quantitative results of the research and the findings that related to the results of the proposed hypotheses and questions were analyzed by using SPSS16 software and analysis of covariance.

FINDINGS

First hypothesis: Narrative therapy is effective in enhancing the children's creativity.

As shown in Table 2, the test statistic is equal to (f: 17.245) that since p <0.01, this value has become significant at the level of one percent. Therefore, there is a significant difference between the level of creativity in the two experimental and control group in posttest after controlling the effect of pretest. Therefore, narrative therapy had a significant impact on increasing children's creativity at the level of one percent.

Second hypothesis: Narrative therapy is effective in enhancing social development of children.

As shown in Table 3, the test statistic is equal to (f: 9.325) that since p <0.01, this value has become significant at the level of one percent. Therefore, there is a significant difference between the level of social development in the two experimental and control group in posttest after controlling the effect of pretest. Therefore, narrative therapy had a significant impact on increasing children's social development at the level of one percent.

DISCUSSION AND CONCLUSION

As the first hypothesis of the study showed that the narrative therapy is effective in increasing children's creativity, in a way that the results of this test showed that there is a significant difference between the mean score in the pre-test and post-test. These findings of this study are consistent with the research results of the Montero K. & Renee (2002), Schneider and Dube (2005), Angus, Levitt and Hardtke (1999), Yousefi Louyeh (2008), MirKamali and Khorshidi (2009), Jamali et al. (2010), Moghtaderi et al. (2010), Sourtiji et al. (2011) that all had addressed the impact of narrative therapy in increasing children's creativity and the second hypothesis of the study showed that narrative therapy is effective in increasing children's social development. These findings of this study are consistent with the research results of the Montero K. & Renee (2002), Schneider and Dube (2005), Angus, Levitt and Hardtke (1999), Moghtaderi et al. (2010), Asgharzadeh et al. (2011), Sanaat Negar et al (2012) and Rajabi (2012) that all had addressed the impact of narrative therapy in increasing children's social development. Vygotesky also believed that child's cognitive development mainly depends on the people who live in his world. Knowledge, ideas, attitudes and values of the individuals are evolved through interaction with others. According to him, by considering the fact that the parents have more relationships with their children, they can have an extraordinary impact on social development of their children (Seif, 2010). According to “Parsons”, socialization process takes place essentially through the mechanisms of generalization, imitation and replication that are very important. Processes of motivation that enter into the learning mechanisms are organized as a part of the socializing mechanisms through the participation of children in a complementary system of role expectations (Parsons, 1992). Several factors are effective in the development of creativity and social development of children. Story and storytelling is as old as human history, in such a way that human has illustrated his life within the framework of stories and the stories have also changed and transformed the human life. Stories are representation of us and our experiences (Asgharzadeh, 2011). Life is a continuous process of disciplining and organizing experiences. When there is no complete understanding of the world, the need to attribute the meaning to the experiences is felt more than ever. Thus, long-life experiences and events of each person becomes a story. When people are telling stories, they can discover meaningful sequences and relationships and gain deeper insight into their condition. In fact, the stories, as fictional and cultural structures, possess significant and outstanding points in individual and social terms (Asgharzadeh, 2011). Storytelling is an activity which encompasses representation of self, the world and the various characters that individuals interact with them and thus forms their relationships with others. In fact, the stories provide a framework for self-creation and also enhancing the self-understanding and effectiveness of interpersonal relationships (DiMaggio et al, 2003 quoted by Yousefi Louyeh, 2006). Given that the results showed that the narrative therapy is effective in enhancing the creativity and social development of children, according to this basis, following recommendations are presented for implementing the

narrative therapy, among children and accordingly to increase their creativity and social development. Pre-school centers should involve narrative therapy, as the part of their basic programs, in education and training of the children. Parents should try to encourage their children to read stories. In-service trainings must be held in order to instruct the school teachers about the narrative therapy practices in education centers. Through the mass media, parents should be encouraged to read stories to their children.

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Table 1: Package of narrative therapy

Narrative therapy sessions	Activities	Objectives
First session	Stating objectives of the training sessions and implementation of the pre-test	Becoming familiar with the participants
Second session	Telling the story of *Kerm-e Se Noghte*(Worm with three points)	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Third session	Reviewing the assignments of previous session and telling the story of Bargaha va Sangha (Leaves and Rocks)	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Fourth session	Reviewing the assignments of previous session and telling the story of What Do You Do with a Tail Like This?	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Fifth Session	Reviewing the assignments of previous session and telling the story of *Mah-e man….Mah-e ma* (My Moon…Our Moon)	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Sixth session	Reviewing the assignments of previous session and telling the story of Large pines and small Oaks	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Seventh Session	Reviewing the assignments of previous session and telling the story of *Safar Kaleh Gondeh* (Big-headed Safar)	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Eighth Session	Reviewing the assignments of previous session and telling the story of *Harf Abad*	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Ninth session	Reviewing the assignments of previous session and telling the story of *Kuti Kuti*	Promoting perspectives and perceptions of children about the creativity and social development and provision of exercises
Tenth session	Evaluation of the leanings of previous sessions and implementation of posttest	

Table 2: Covariance analysis of the impact of narrative therapy in enhancing the children's creativity

Source of changes	Total of squares	Degree of freedom	Mean square	Test statistic	p	ETA	Statistical power
The effect of pre-test	326.748	1	34.587	25.574	0.00	0.845	0.657
The effect of independent variable	230.458	1	252.657	17.245	0.00	0.745	0.758

Error	398.398	27	13.354				
Adjusted total	878.278	29					

Table 3: Covariance analysis of the impact of narrative therapy in enhancing the children's social development in the control and experimental groups

Source of changes	Total of squares	Degree of freedom	Mean square	Test statistic	p	ETA	Statistical power
The effect of pre-test	31.458	1	32.241	8.542	0.07	0.251	0.897
The effect of independent variable	35.574	1	33.854	9.325	0.005	0.265	0.864
Error	97.357	27	3.658				
Adjusted total	158.568	29					