



RESEARCH ARTICLE

Effect of Nano-Zinc Chelate and Nano-Biofertilizer on Yield and Yield Components of Maize (*Zea mays* L.), Under Water Stress Condition

Amin Farnia^{1*} and Mohammad Mehdi Omid²

¹Assistant professor of department of agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran

²M.Sc student of department of agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.

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*Address for correspondence

Amin Farnia

Assistant professor of department of agriculture,
Boroujerd branch, Islamic Azad University,
Boroujerd, Iran.

E-mail : Aminfarnia@yahoo.com



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ABSTRACT

This experiment was laid out in order to determine the effect of nano-Zinc chelate and nano-biofertilizer on yield and yield components of maize under water stress condition on a loam clay soil, in Islamic Azad University of Boroujerd, Iran, during the growing seasons 2013- 2014. The experiment was laid out in a split-factorial design based on randomized block design with three replications. The experiment was laid out in a split-factorial design based on randomized block design with three replications. Treatments were different irrigation periods (7, 14 and 21 days) in main plots and nano-Zinc chelate and nano-biofertilizer in two levels of use and non use of them as factorial in sub plots. The results showed that the effects of water stress, nano-Zn and nano-biofertilizer treatments on all traits were significant. The comparison of the mean values showed that 7 day irrigation period treatment with use of nano biofertilizer and nano-Zn had the highest chlorophyll SPAD measurement, 100grain weight and harvest index. Application of Zn nanofertilizer and nano biofertilizer treatment had the highest and control treatment had the lowest biomass yield. Also, 7 day irrigation period treatment with use of nano-biofertilizer had the highest number of row per cob, number of grain per cob and grain yield but 21 day irrigation period treatment without use of nano-biofertilizer had the lowest of them. However, 7day irrigation period treatment with use of Zn nanofertilizer had the highest grain yield and non application of Zn nanofertilizer in 21 day irrigation period treatment had the lowest of grain yield. However, the present study concluded that maximum production of maize was recorded for normal irrigation as 7 day irrigation period and application of nano- Zn nutrient and nanobiofertilizer nutrient, while severe water stress without

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application of nano- Zn nutrient and nanobiofertilizer produced minimum production. Therefore, we can increase yield and yield components of maize by decrease of irrigation period and application of nano- Zn nutrient and nanobiofertilizer as nutrient.

Key words: Biofertilizer, maize, nano-Zn and grain yield

INTRODUCTION

A lot of cereals growing areas are located in arid and semi-arid regions where nutrient deficiency exists due to high soil pH, free calcium carbonate and low organic matter, drought and salt stresses, imbalanced application of NPK fertilizers and high bicarbonate content of irrigation water (Narimani et al., 2010; Ali, 2012). Maize (*Zea mays* L.) is one of the major cereal crops and is a very versatile grain that benefits mankind in many ways. It is a versatile crop and ranks third following wheat and rice in world production as reported by Food and Agriculture Organization the most important cereal crops in the world. It is a versatile crop and ranks third following wheat and rice in world production as reported (FAO, 2002).

Performance reduction caused by environmental stresses such as drought and element deficiencies is a serious problem. In the U.S. 75 percent of agriculture are potentially faced with these tensions (Bennet and Creen, 1991). Several studies have also shown that optimum yield can be obtained with irrigation at branching, flowering and pod formation stages (Prihar and Sandhu, 1968). Water stress is deleterious for plant growth, yield and mineral nutrition (Garg et al., 2004; Samarah et al., 2004). Soil moisture status during the reproductive phase of crops plays an important role to determine the impact of yield component in final grain yield (Singh and Bhushan, 1980). Biglouei et al (2007) reported that the increase of drought stress in K.S.C.704 corn, led to increase of grain yield and protein percentage. They also declared that grain protein in irrigation treatments after 50, 75 and 100 percent water depletion, were relatively 5.8, 7.2 and 7.4. The studies of researchers have indicated that, lack of organic materials and existence of alkali reaction in calcareous soils can lead to lack of micronutrients in these soils (Auge, 2001).

Zinc is an essential element for plants and animals and plays an important role in plants metabolic system. This element activates enzymes and involved in protein, lipids, carbohydrates and nucleic acid metabolism (Khan et al., 2002; Zlatimira, 2002). Nearly 200 enzymes and transcription elements of zinc need it as one of the most essential components. Zinc plays an important role in protein and carbohydrates syntheses. It also has effects on growth of stem and root (Kabata-Pendias, 1999). Zinc has a major role in cell defenses against ROS and as a protective factor against several chemical compositions of oxidation such as membrane lipids, protein, chlorophyll, and enzyme having SH and DNA (Cakmak, 2000). Zinc plays an important role as a metal component of enzymes (alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural, or regulator cofactor of a large number of enzymes (Marschner, 1986). Zinc also plays a key role in controlling the production and toxicity of free radicals that can damage membrane lipids and sulphhydryl groups (Alloway, 2004). Soleimani (2006) reported increase in biological yield for foliar application of zinc. Marshner (1993) reported that, by increasing consumption of Iron and zinc in corn, we can witness that the total amount of carbohydrate and grain protein is increased, and as a result the gain weight, number of grains and at last yield will be increased as well. Tahmasebi et al (2003) declared that by increasing amounts of zinc, absorption of nitrogen will be decreased by the plant, yet potassium absorption will be increased.

Nanotechnology such as using nano-scale fertilizer particles may offer new techniques in improving existing crop management (Ghafari and Razmjoo, 2013). This technology is particularly applied in chelate fertilizers such as zinc chelate. The present study aims at investigating the impact of zinc chelate fertilizer in two forms of nanochelate and non-nanochelate on growth and some biochemical processes of cotton plant (Rezaei and abbasi, 2014). Effect of nano oxide iron alone or with iron chelate and sulphate on wheat production and grain quality especially Fe content has



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not been compared (Ghafari and Razmjoo, 2013). In addition, there is a little information on the accumulation of antioxidant enzymes and their possible role on yield and quality of wheat under nano oxide iron, iron chelate, and iron sulphate application (Ghafari and Razmjoo, 2013). Liu et al. (2005) reported that nano-fertilizers promoted the growth and photosynthesis of peanut. Sheykhbaglou et al. (2010) showed that application of nanofertilizers such as nano-iron oxide particles increased soybean yield. Prasad et al. (2012) reported that nano-scale zinc oxide particles increased stem and root growth and pod yield of peanut as compared with ZnSO₄ application.

Therefore this study was planned to examine effect of Nano-Zinc chelate and Nano-biofertilizer on yield and yield components of maize (*zea mays L.*) SC600 cultivar under water stress condition.

MATERIALS AND METHODS

Field material and Experimental design

This study was carried out in order to evaluate the effect of nano-Zinc chelate and nano-biofertilizer on yield and yield components of maize (*Zea mays L.*) SC600 cultivar under water stress condition in the faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch (experiment station: Hamedan), Iran during the growing seasons 2013- 2014. Soil property of experimental field showed in table1.

Treatments

The experiment was laid out in a split-factorial design based on randomized block design with three replications. Treatments were different irrigation periods (7, 14 and 21 days) in main plots and Nano-Zinc chelate and Nano-biofertilizer in two levels of use and non use of them as factorial in sub plots. Foliar application of Nano-Zinc chelate treatment was apply in two stages (4 -6 leaf and before of flowering).

Yield and yield components determination

In this field experiment there were 6 rows in each plots and rows were 6 m long with 0.75 m row spacing and plant to plant spacing was 18 cm too. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle 3m² of the four central rows were harvested. After irrigation SPAD was recorded by manual chlorophyll meter. 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 3m² of the four central rows in each plot. 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 harvest index was accounted with follow:

$$HI = (\text{Economical yield} / \text{Biological yield})$$

Statistical analysis

The statistical analyses to determine the individual and interactive effects of treatments were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.





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RESULTS AND DISCUSSION

Chlorophyll SPAD: The analysis of variance showed that, the effect of water stress and Zn Nanofertilizer, Nano biofertilizer and interaction between them on chlorophyll SPAD were significant only (table 2). The comparison of the mean values of the chlorophyll SPAD showed that 7 day irrigation period treatment with use of Nano biofertilizer and Nano-Zn had the highest (55) and 21 day irrigation period treatment without Nano biofertilizer and Nano-Zn had the lowest (30) chlorophyll SPAD measurement (figure 1).

Number of row per cob: The effect of water stress and Zn Nanofertilizer, Nano biofertilizer and interaction between water stress and Nano biofertilizer on number of row per cob was significant (table 2). The comparison of the mean values of the number of row per cob for interaction between water stress and Nano biofertilizer showed that 7 day irrigation period treatment with use of Nano-biofertilizer had the highest (17) number of row per cob and 21 day irrigation period treatment without use of Nano-biofertilizer had the lowest (12) number of row per cob (figure 2).

Number of grain per cob: The effect of water stress and Zn Nanofertilizer, Nano biofertilizer and interaction between water stress and Nano biofertilizer on number of grain per cob were significant (table 2). The comparison of the mean values of the number of grain per cob for interaction between water stress and Nano biofertilizer showed that 7 day irrigation period treatment with use of Nano biofertilizer had the highest (520) number of grain per cob and 21 day irrigation period treatment without use of Nano biofertilizer had the lowest (280) number of grain per cob (figure 3).

100 grain weight: The effect of water stress and Zn Nanofertilizer, Nano biofertilizer, interaction between water stress and Nano biofertilizer and three side interaction on 100-grain weight were significant at 1% level (table 2). The comparison of the mean values of the 100-grain weight showed that 7 day irrigation period treatment with use of Nano biofertilizer and Nano-Zn had the highest (27g) and 21 day irrigation period treatment without Nano biofertilizer and Nano-Zn had the lowest (16g) 100-grain weight (figure 4).

Biomass yield : The effect of water stress and Zn Nanofertilizer, Nano biofertilizer and interaction between Zn Nanofertilizer and Nano biofertilizer on biomass yield were significant (table 2). The comparison of the mean values of the biomass yield for interaction between Zn Nanofertilizer and Nano biofertilizer showed that used of Zn Nanofertilizer and Nano biofertilizer treatment had the highest (17000kg.ha⁻¹) biomass yield and control treatment had the lowest (11000kg.ha⁻¹) biomass yield (figure 5).

Grain yield: The results showed that the effect of water stress and Zn Nanofertilizer, Nano biofertilizer and interaction between water stress with Zn Nanofertilizer and Nano biofertilizer on grain yield were significant (table 2). The comparison of the mean values of the grain yield for interaction between water stress and Nano biofertilizer showed that 7day irrigation period treatment with use of Nano biofertilizer had the highest (11.5ton.ha⁻¹) grain yield and non application of Nano biofertilizer in 21 day irrigation period treatment had the lowest (3.2ton.ha⁻¹) grain yield (figure 6). However, the comparison of the mean values of the grain yield for interaction between water stress and Zn Nanofertilizer showed that 7day irrigation period treatment with use of Zn Nanofertilizer had the highest (10.5ton.ha⁻¹) grain yield and non application of Zn Nanofertilizer in 21 day irrigation period treatment had the lowest (2.7ton.ha⁻¹) grain yield (figure 7).

Harvest index (HI): The analysis of variance showed that, the effect of water stress and Zn Nanofertilizer, Nano biofertilizer and interaction between them on harvest index were significant (table 2). The comparison of the mean values of the harvest index showed that 7 day irrigation period treatment with use of Nano biofertilizer and Nano-Zn had the highest (51%) and 21 day irrigation period treatment without Nano biofertilizer and Nano-Zn had the lowest (28%) harvest index (figure 8).



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In this study the results revealed that the effect of water stress nanobiofertilizer and nano-Zn on all traits were significant (table 2). In general, 7day irrigation period and application of nanobiofertilizer and nano-Zn produced the highest chlorophyll contents (SPAD) (figure 1). Nano-Zn application increased total chlorophyll contents as compared with the control, but there was marked differences between Zn rates on these traits. That was perhaps due to the association of Zn with chlorophyll formation (Mazaherinia et al., 2010). In line with our results, Liu et al. (2005) reported that nanofertilizers such as nano-Fe₂O₃ application increased chlorophyll content of peanut and Amanullah et al. (2012) showed that application of iron sulphate in soil and foliar spray increased chlorophyll content of maize leaf. Increased in chlorophyll content of wheat in our experiment could be due to promotion of the absorption and utilization of nutrients such as nitrogen by nano-fertilizers compound as concluded by Liu et al. (2005).

In addition, Sheykhbaglou et al. (2010) reported that nano fertilizers increased pod and leaf dry weight and yield of soybean, but had no effects on plant height and other growth and yield parameters. Their results were in general agreement with ours. In line with our results, Sheykhbaglou et al. (2010) showed that application of nano-fertilizers increased soybean yield.

In line with our results, Habib (2009) reported that application of 150 g ha⁻¹ Fe as Fe₂O₃ increased wheat grain yield. Zeidan et al. (2010) reported that application of 1% FeSO₄ increased yield and yield components of wheat.

The maximum amount of yield and its components were observed in treatments of 7 day irrigation period and application of Zinc nano-chelate and nanobiofertilizer. Rezaei and abbasi (2014) founded that the application of zinc chelate and specially the nano-chelate of zinc can be considered as a measure for cotton crop improvement by increasing weight of 20 bolls and number of bolls per plant. They also told that maximum height, fresh weight, and dry weight were obtained in treatments with nano-chelate and chelate of zinc and there were no significant difference in squaring, squaring-flowering, and flowering application stages and application of chelate and nano-chelate zinc fertilizer in the present study improved physiological processes in cotton as it increased the amount of chlorophyll and antioxidant activity of peroxidase, catalase, and polyphenol oxidase as a result of increase in fresh and dry weight.

In the present study water stress in 21 day irrigation period decreased grain yield but application of nanobiofertilizer and Zn-nanofertilizer increased grain yield of soybean. Soil moisture status during the reproductive phase of crops plays an important role to determine the impact of yield component in final grain yield (Singh and Bhushan, 1980). The reduction in grain yield under water stress treatments may be attributed to the limitation of dry matter partitioning to the reproductive sink or even grain formation factors as has been reported by Turk et al (1980). The number of grain per plant in 7 day irrigation period giving over the 21 day irrigation period. However, the results showed that under 7 day irrigation period conditions and application of bio and nano fertilizers significantly gave better grain yields than 21 day irrigation period conditions. Thalooth et al (2006) reported that zinc spraying under water stress conditions, had positive effect on growth, yield, and yield components of plants. The findings of this experiment conformed to the results obtained by Sheykhbagloo et al (2009). Pandey et al (2002) stated that the maximum water consumption by the corn is almost when silk rating or immediately after that. Application of nano-Zn nutrient and nanobiofertilizer had a positive effect on the grain yield and biomass yield and yield components of maize. In maize, the final grain yield is dependent on the number of cob per plant, number of grains per cob and the extent to which grains are filled. In the present study, the reduction in grain yield under water stress and non application of nano- Zn nutrient and nanobiofertilizer were associated with dramatic decrease in all yield components. Supporting evidences were reported by many researchers (Ludlow and Mushow, 1990). Decrease biomass yield under lower soil moisture might be due to reduction of leaf area and photosynthesis rate (Sinaki et al., 2007). The biomass yield in the 7 day irrigation period and application of nano- Zn nutrient and nanobiofertilizer treatment had a 15% over the non application of any nutrient and 21 day irrigation period treatment (figure 5). Latiri-Soki et al (1998) reported that, irrigation and fertilizers increased biomass yield and grain yield.





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In the present study harvest index was dramatically decreased with increased in irrigation period and application of nano- Zn nutrient and nanobiofertilizer treatments. Ziska and Hall (1983) founded that the effect of water stress on HI to the reduction in assimilate supply attributed. The present study concluded that maximum production of maize (grain yield, yield components and grain yield) was recorded for normal irrigation as 7 day irrigation period and application of nano- Zn nutrient and nanobiofertilizer nutrient, while severe water stress and non application of nano- Zn nutrient and nanobiofertilizer produced minimum production. Therefore, we can increase yield and yield components of maize by decrease of irrigation period and application of nano- Zn nutrient and nanobiofertilizer as nutrient.

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Table 1. Soil property of experiment site.

soil Texture	sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (ppm)	N (%)	pH	EC (ds/m)	Depth
LC	20	45	35	220	8.2	0.1	7.7	0.409	0-30

Table2. Analysis of variance for yield, yield components of maize under water stress , Nanobiofertilizers and Nano-Zn nutrient

treatments	df	SPAD	number of row per cob	number of grain per cob	100 grain weight	biomass yield	grain yield	harvest index
R	2	8.7	4.35	2219	0.33	5.8	0.76	13.6
Irrigation (A)	2	472**	37.9**	152412**	329**	232**	152**	1932**
Ea	4	4.9	1.8	718	2.4	2	0.42	61
Nanobiofertilizer (B)	1	414**	30.1**	32669**	87**	123**	49**	363**
Nano-Zn ©	1	103**	11.9**	19778**	20**	21**	19**	291**
A*B	2	2.8	4.3*	4540**	6.6*	0.44	6.4**	125*
A*C	2	0.55	0.43	849	2.9	0.76	2.5*	39
B*C	1	0.42	0.13	301	2.8	5.2*	0.01	34
A*B*C	2	28.9*	0.63	879	4.8*	0.52	0.6	84*
Eb	18	6.1	0.91	545	1.2	0.91	0.47	23
CV(%)		5.7	7	5.4	5.9	6.3	12.5	13.8

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





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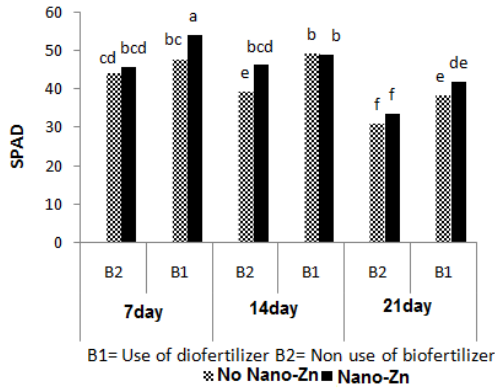


Figure 1. Effect water stress, biofertilizer and Zn nutrient on chlorophyll SPAD in maize. Means by the uncommon letter in each column are significantly different (p<0.05)

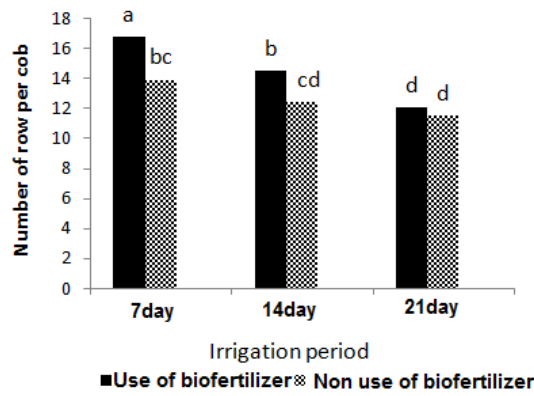


Figure 2. Effect water stress and biofertilizer on number of row per cob in maize. Means by the uncommon letter in each column are significantly different (p<0.05)

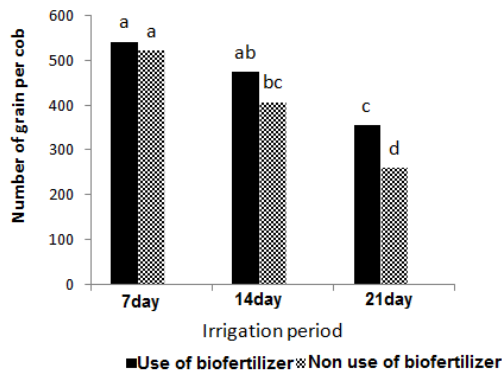


Figure 3. Effect water stress and biofertilizer on number of grain per plant in maize. Means by the uncommon letter in each column are significantly different (p<0.05)





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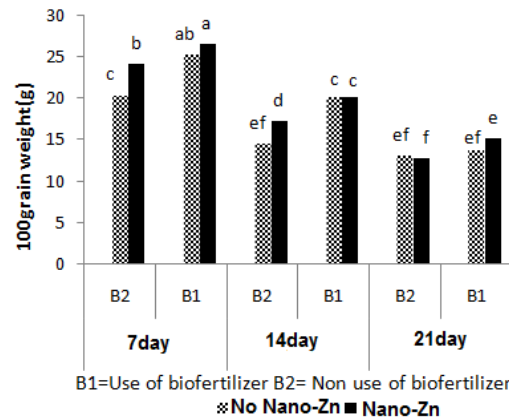


Figure 4. Effect water stress, biofertilizer and Zn nutrient on 100grain weight in maize. Means by the uncommon letter in each column are significantly different ($p < 0.05$)

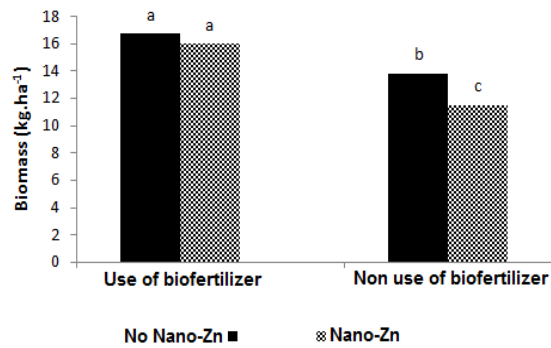


Figure 5. Effect biofertilizer and Zn nutrient on biomass yield of maize. Means by the uncommon letter in each column are significantly different ($p < 0.05$)

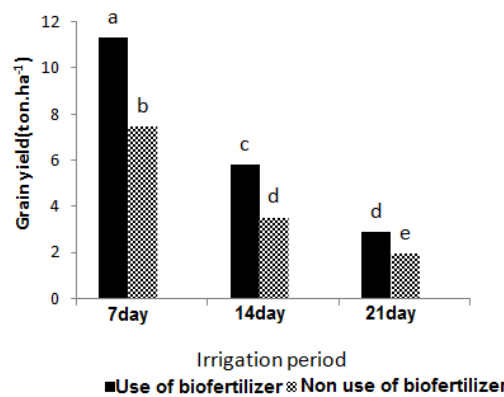
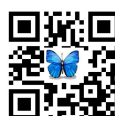


Figure 6. Effect water stress and biofertilizer on grain yield of maize. Means by the uncommon letter in each column are significantly different ($p < 0.05$)





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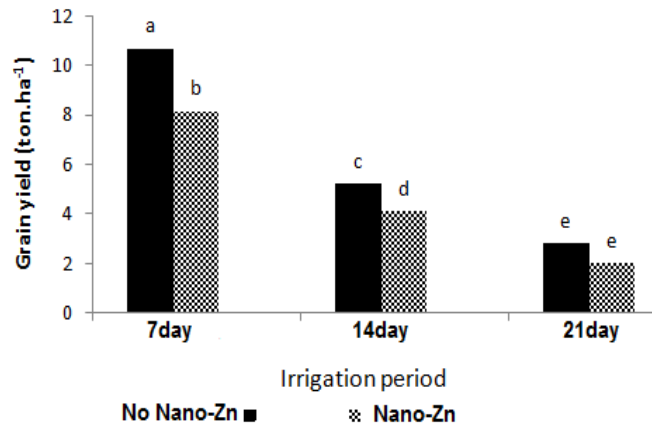


Figure 7. Effect water stress and Zn nutrient on grain yield of maize. Means by the uncommon letter in each column are significantly different (p<0.05)

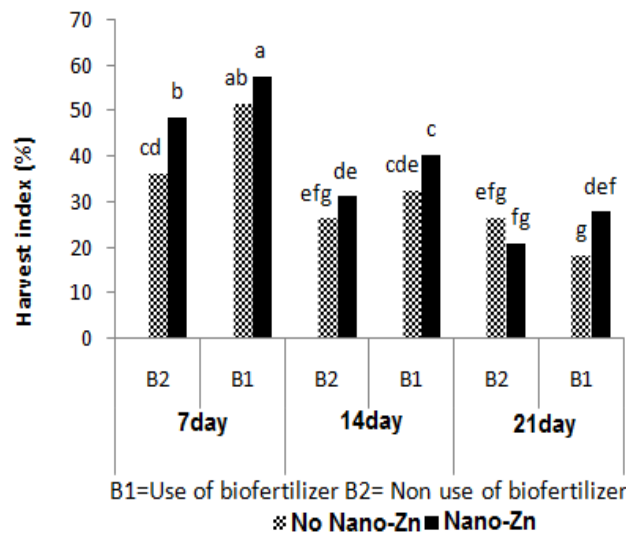


Figure 8. Effect water stress, biofertilizer and Zn nutrient on harvest index in maize. Means by the uncommon letter in each column are significantly different (p<0.05)





Response of Maize (*Zea mays* L.) to Foliar Application of Zinc Nutrient under Water Stress Condition

Amin Farnia^{1*} and Mohammad Amir Feyzi²

¹Assistant professor of department of agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.

²M.Sc student of department of agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.

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*Address for correspondence

Amin Farnia

Assistant professor of department of agriculture,

Boroujerd branch,

Islamic Azad University,

Boroujerd, Iran.

E-mail : Aminfarnia@yahoo.com



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ABSTRACT

To determine the effects of water stress and foliar application of Zinc nutrient on yield and yield components of maize a field experiment was conducted on a loam clay soil, in Islamic Azad University of Boroujerd, Iran, during the growing seasons 2013- 2014. The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were different irrigation periods (7, 14 and 21 days) in main plots and foliar application of Zn sulphate, Zn chelate, water and non application of them in sub plots. Foliar use of treatment was apply in two stages (6 leaf and before of flowering). The results of present study reviled that the effects of water stress and Zn nutrient treatments on all traits were significant but the interaction effect of them on 100grain weight and biomass and grain yield were significant only. Simple mean comparison of traits showed that 7 day irrigation period and foliar application of Zn sulphate treatments had the highest plant height, number of row per cob, number of grain per cob and harvest index and 21 day irrigation period and non-application of any Zn and water treatment had the lowest of them. However, highest 100-grain weight, grain yield and biomass yield were founded in foliar application of Zn nutrient in 7 day irrigation period and lowest of them were founded at non-application of any Zn and water treatment in 21 day irrigation period. The present study concluded that maximum production of maize was recorded for non stress treatment and was followed by application of foliar application of Zn nutrient, while severe water stress produced minimum production. Also, results of these experimnet showed that application of Zn fertilizer had better effect on

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grain yield, yield components compared to the control. However, application of Zn sulphate that had highest production of maize is better than Zn chelate and water. Therefore, we can increase yield and yield components of maize by decrease of irrigation period and foliar application of Zn sulphate and Zn chelate spacialy Zn sulphate.

Key words: Irrigation, maize, water stress and Zn sulphate

INTRODUCTION

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

Drought stress causes deceleration of cell enlargement and thus reduces stem length by inhibiting inter nodal elongation and also checks the tillering capacity of plants (Ashraf and O'Leary, 1996). Several studies have also shown that optimum yield can be obtained with irrigation at branching, flowering and pod formation stages (Prihar and Sandhu, 1968). Dry stress may cause some problems in absorbing process or the process of nutrient accumulation, this can not only lead to fertilizer loss but also causes decrease in yield of grain and plants (Chogan et al, 2004). Biglouei et al (2007) reported that the increase of drought stress in K.S.C.704 corn, led to increase of grain yield and protein percentage. They also declared that grain protein in irrigation treatments after 50, 75 and 100 percent water depletion, were relatively 5.8, 7.2 and 7.4. The studies of researchers have indicated that, lack of organic materials and existence of alkali reaction in calcareous soils can lead to lack of micronutrients in these soils (Auge, 2001).

Zinc is considered to be one of the smallest nutritious elements for many organisms. Nearly 200 enzymes and transcription elements of zinc need it as one of the most essential components. Zinc plays an important role in protein and carbohydrates syntheses. It also has effects on growth of stem and root (Kabata-Pendias, 1999). Zinc plays an important role as a metal component of enzymes (alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural, or regulator cofactor of a large number of enzymes (Marschner, 1986). Mahady (1990) found that foliar application of Zn SO for faba bean plants increased number of pods/plant and seed yield/fed. Guenis et al. (2003) and Soleimani (2006) reported marked increase in number of grains spike-1 of wheat for foliar application of boron and zinc, respectively. Soleimani (2006) reported increase in biological yield for foliar application of zinc. Torun et al. (2001) and Grewal et al. (1997) reported increased wheat production with application of zinc and boron over control. Grain protein content and baking quality highly depend on genetic background and environmental factors, especially influence of drought and heat stress, during the grain filling period (Luo et al., 2000; Ottman et al., 2000). Marshner (1993) reported that, by increasing consumption of Iron and zinc in corn, we can witness that the total amount of carbohydrate and grain protein is increased, and as a result the gain weight, number of grains and at last yield will be increased as well. Tahmasebi et al (2003) declared that by increasing amounts of zinc, absorption of nitrogen will be decreased by the plant, yet potassium absorption will be increased. They also concluded that the most balanced status between nitrogen, phosphor and potassium absorption with 20 kilograms zinc sulfate per hectare was observed in saline soils. Fecencko and Lozek (1998) studied the effect of zinc sources on yield, protein percentage and macronutrient absorption in corn, and they witnessed that by three years of manure, the average amount of grain protein was significantly increased. Therefore this study was planned to examine effect of drought stress and Zinc fertilizer on yield and yield components of maize.





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MATERIALS AND METHODS

Field material and Experimental design

This study was carried out in order to evaluate the effects of drought stress and Zinc fertilizer on yield and yield components of maize in the faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch (experiment station: Hamedan), Iran during the growing seasons 2013- 2014. Soil property of experimental field showed in table1.

Treatments

The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were different irrigation periods (7, 14 and 21 days) in main plots and foliar application of Zn sulphate, Zn chelate, water and non application of them in sub plots. Foliar use of treatment was apply in two stages (6 leaf and before of flowering).

Yield and yield components determination

In this field experiment there were 6 rows in each plots and rows were 6 m long with 0.75 m row spacing and plant to plant spacing was 18 cm too. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle 3m² of the four 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 3m² of the four central rows in each plot. 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 harvest index was accounted with follow:

HI = (Economical yield / Biological yield)

Statistical analysis

The statistical analyses to determine the individual and interactive effects of treatments were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

RESULTS AND DISCUSSION

Plant height: The analysis of variance showed that, the effect of water stress and Zn nutrient on plant height were significant only (table 2). The comparison of the mean values of the plant height showed that 7 day irrigation period treatment had the highest (210cm) plant height and 21 day irrigation period treatment had the lowest (160cm) plant height. In foliar application of Zn treatments Zn sulphate treatment had the highest (195cm) plant height and control treatment had the lowest (176cm) plant height and the differences were significant (table 3).

Number of row per cob: The effect of water stress and Zn nutrient on number of row per cob was significant (table 2). The comparison of the mean values of the number of row per cob for water stress showed that 7 and 14 day irrigation period treatments had the highest (16) number of row per cob and 21 day irrigation period treatment had the lowest (13) number of row per cob. In foliar application of Zn treatments Zn sulphate treatment had the highest



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(16) number of row per cob and control treatment had the lowest (13) number of row per cob and the differences were significant (table 3).

Number of grain per cob: The effect of water stress and Zn nutrient on number of grain per cob was significant (table 2). The comparison of the mean values of the number of grain per cob for water stress showed that 7 day irrigation period treatment had the highest (500) number of grain per cob and 21 day irrigation period treatment had the lowest (380) number of grain per cob. In foliar application of Zn treatments Zn sulphate treatment had the highest (470) number of grain per cob and control treatment had the lowest (395) number of grain per cob (table 3).

100 grain weight: The results showed that the effect of water stress, Zn nutrient and interaction between them on 100-grain weight were significant at 1% level (table 2). The comparison of the mean values of the 100-grain weight for interaction between water stress and Zn nutrient foliar application Zn sulphate in day irrigation period treatment had the highest (26g) 100-grain weight and non application of Zn in 21 day irrigation period treatment had the lowest (14g) 100-grain weight (figure 1).

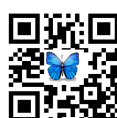
Biomass yield : The effect of water stress, Zn nutrient and interaction between them on biomass yield were significant at 1% level (table 2). The comparison of the mean values of the biomass yield for interaction between water stress and Zn nutrient foliar application Zn sulphate in day irrigation period treatment had the highest (19000kg.ha⁻¹) biomass yield and non application of Zn in 21 day irrigation period treatment had the lowest (9000kg.ha⁻¹) biomass yield (figure 2).

Grain yield: The results showed that the effect of water stress, Zn nutrient and interaction between them on grain yield were significant at 1% level (table 2). The comparison of the mean values of the grain yield for interaction between water stress and Zn nutrient foliar application Zn sulphate in day irrigation period treatment had the highest (10750kg.ha⁻¹) grain yield and non application of Zn in 21 day irrigation period treatment had the lowest (2350kg.ha⁻¹) grain yield (figure 3).

Harvest index (HI): The effect of water stress and Zn nutrient on harvest index was significant (table 2). The comparison of the mean values of the harvest index for water stress showed that 7 day irrigation period treatment had the highest (47%) harvest index and 21 day irrigation period treatment had the lowest (28%) harvest index. In foliar application of Zn treatments Zn sulphate treatment had the highest (44%) harvest index and control treatment had the lowest (31%) harvest index (table 3).

The results of present study revealed that the effect of water stress and foliar application of Zn on all traits were significant but the interaction effect of them on 100grain weight and biomass and grain yield were significant only (table 2). Water stress is deleterious for plant growth, yield and mineral nutrition (Garg et al., 2004; Samarah et al., 2004). Soil moisture status during the reproductive phase of crops plays an important role to determine the impact of yield component in final grain yield (Singh and Bhushan, 1980). This study has showed that maize grain yield and yield components decreased significantly with the increase of irrigation period. The reduction in plant height, number of row per cob and number of grain per cob under water stress treatments may be attributed to the limitation of dry matter partitioning to the reproductive sink or even grain formation factors as has been reported by Turk et al (1980). The significant reduction in number of total grains in plant under drought stress may be attributed to the abscission of the reproductive structures. Ziska and Hall (1983) and Gwathmey and Hall (1992) reported similar results. The number of grain per plant in 7 day irrigation period giving a 22% increase over the 21 day irrigation period.

The grain yield in the water stress condition was restricted by limited moisture availability. Drought occurrence in relation to anthesis stage causes a drastic reduction in yield and yield components (Seghatoleslami et al., 2008). However, the results showed that under 7 day irrigation period conditions significantly gave better grain yields than



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21 day irrigation period conditions. The grain yield of maize in the 7 day irrigation period and application of Zn sulphate treatment had a 10750kg.ha⁻¹ that is 5 equivalent of grain yield in non application of any foliar nutrient and 21 day irrigation period (figure 3). Pandey et al (2002) stated that the maximum water consumption by the corn is almost when silk rating or immediately after that. Water deficit when tasselling and silk rating along with decreased grain number per ear decreases grain yield the most. Therefore, the main cause of decreased grain yield in drought stress treatments seemed to be significant decrease in grain number per cob. Thalooh et al (2006) reported that zinc spraying under water stress conditions, had positive effect on growth, yield, and yield components of plants. The findings of this experiment conformed to the results obtained by Sheykhbagloo et al (2009). This result was also supported by the other researchers (Mojadam, 2006 and Lack, 2006).

Folier application of Zn nutrient had a positive effect on the grain yield and biomass yield and yield components of maize. In maize, the final grain yield is dependent on the number of cob per plant, number of grains per cob and the extent to which grains are filled. In the present study, the reduction in grain yield under water stress was associated with dramatic decrease in all yield components (Table 3). Supporting evidences were reported by many researchers (Ziska and Hall, 1983; Ludlow and Mushow, 1990; Gwathmey et al., 1992). They attributed the reduction in grain yield under water stress to the reduction in number of grain per plant, biomass yield and grain weight. Turk and Hall (1980) attributed the reduction in grain yield under drought stress to the secondary detrimental effects of drought avoidance on CO₂ assimilation. This result suggests that maize exhibit reproductive plasticity under drought stress conditions.

Decrease biomass yield under lower soil moisture might be due to reduction of leaf area and photosynthesis rate (Sinaki et al., 2007). Different irrigation period treatments indicate with increasing water stress the biomass yield decreased significantly. The biomass yield in the 7 day irrigation period and application of Zn sulphate treatment had a 50% over the non application of any foliar nutrient and 21 day irrigation period treatment (figure 2). Latiri-Soki et al (1998) reported that, irrigation and fertilizers increased biomass yield and grain yield. They suggested the increase might be due to increased leaf area index (LAI) and an increase in the period for which the crop remained green which resulted in increased capture efficiency of radiation energy and consequently more dry matter production.

In the present study harvest index was dramatically decreased with increased in irrigation period and non application of any Zn nutrient treatments. Also, Ziska and Hall (1983) founded that the effect of water stress on HI to the reduction in assimilate supply attributed. This suggests that maize which application of Zn nutrient gave higher grain yield under water-stressed conditions could play an important role in sustaining crop production in semi arid regions.

CONCLUSION

The present study concluded that maximum production of maize (grain yield, yield components and grain yield) was recorded for non stress treatment and was followed by application of foliar application of Zn nutrient, while severe water stress produced minimum production. Also, results of these experiment showed that application of Zn fertilizer had better effect on grain yield, yield components compared to the control. However, application of Zn sulphate that had highest production of maize (grain yield, 100 grain weight and grain protein yield) is better than Zn chelate and water. Therefore, we can increase yield and yield components of maize by decrease of irrigation period and foliar application of Zn sulphate and Zn chelate spacialy Zn sulphate.





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Table 1. Soil property of experiment site.

soil Texture	sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (ppm)	N (%)	pH	EC (ds/m)	Depth
LC	20	45	35	220	8.2	0.1	7.7	0.409	0-30





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Table2. Analysis of variance (mean squares) for yield, yield components of maize under water stress and Zn nutrient

treatments	df	Plant height	number of row per cob	number of grain per cob	100 grain weight	biomass yield	grain yield	harvest index
R	2	1229	1.39	2472	0.18	8608201	737705	108
Irrigation (A)	2	8138**	33**	78257**	381**	213078163**	136727622**	2403**
Ea	4	209	0.35	3945	10.6	2556246	1656643	33
Zn (B)	3	371**	17.1**	12541**	38**	40293589**	23273883**	274**
A*B	6	68	0.82	960	1.7**	2275190*	1426592**	43
Eb	18	56	0.83	1319	0.49	802402	342387	27
CV(%)		4	5.9	8.3	3.88	6.4	10.3	13

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

Table 3. Simple mean comparison for yield, yield components of maize under water stress and Zn nutrient

treatment period	Plant height (cm)	number of row per cob	number of grain per cob	harvest index (%)
Irrigation period				
7 day	210a	16a	500a	47a
14 day	190b	16a	440b	41b
21 day	160c	13b	380c	28c
Zn				
Zn sulphate	195a	16a	470a	44a
Zn chelate	190ab	15a	460a	41a
water	184bc	14b	420b	33b
non application	176c	13b	395b	31b

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

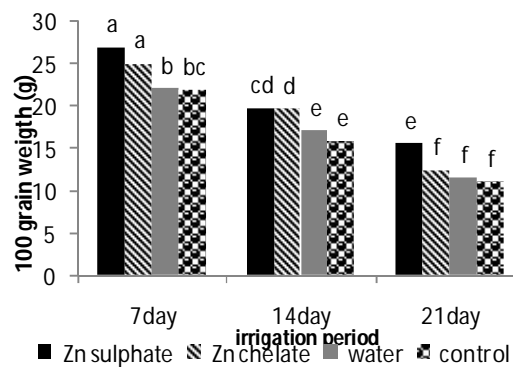


Figure 1. Effect water stress and Zn nutrient on 100 grain weight in maize.

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





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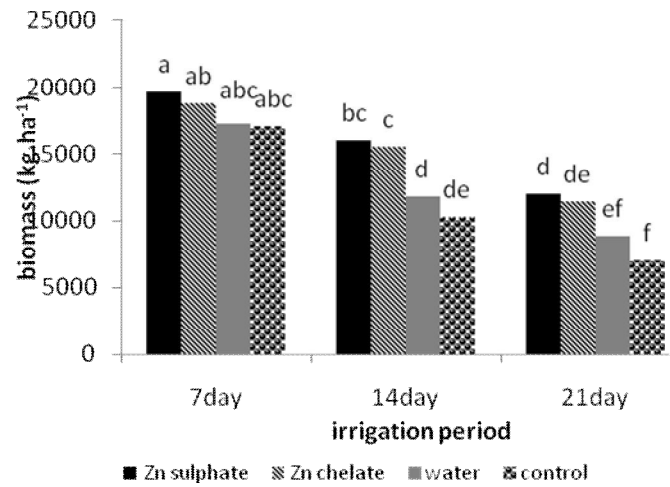


Figure 2. Effect water stress and Zn nutrient on biomass yield in maize.
Means by the uncommon letter in each column are significantly different (p<0.05)

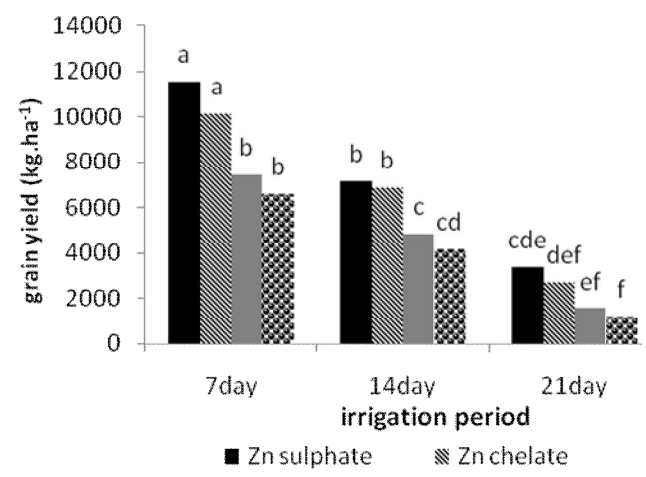


Figure 3. Effect water stress and Zn nutrient on grain yield in maize.
Means by the uncommon letter in each column are significantly different (p<0.05)





RESEARCH ARTICLE

Identification of Natural Enemies of Corn Stem Borer of *Ostrinia Nubilalis* (Hubner) on Four Corn Hybrids in Moghan Region

Javad Salmani Moghanlou¹, Seyed Ali Asghar Fathi¹, Gadir Nuri Ganbalani¹, Jebraeil Razmjou¹, Masoud Taghizadeh¹, Abasalt Rostami Ajirlou² and Morad Shaaban^{3*}

¹ Department of Entomology, School of Agriculture Science, Mohaghegh Ardabili University, Ardabil, Iran

² Young Researchers and Elite Club, Parsabad branch, Islamic Azad University, Parsabad, Iran

³ Young Researchers and Elite Club, Boroujed branch, Islamic Azad University, Boroujerd, Iran.

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*Address for correspondence

Morad Shaaban

Young Researchers and Elite Club,
Boroujed branch, Islamic Azad University,
Boroujerd, Iran.

E-mail : Shaabanm14125@gmail.com



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ABSTRACT

One of the most important insect pests of corn, (*Zea mays* L.,) crop in Moghan region is European corn borer, *Ostrinia nubilalis* (Hübner). The experiment was conducted in a completely randomized block design with four replications. The predators and parasitoids of the European corn borer were collected and identified on four corn hybrids in an experimental field. The seeds of corn hybrids were planted in an experimental field, approximately 700 m². Samplings were conducted from the V6 (six leaf collar) until the R3 (milk) growth stages of corn. On each sample date, five plants/hybrid from each of the four replication were selected randomly and number of eggs (parasitized and non- parasitized) of *O. nubilalis* per plant was recorded using a 20X hand lens, then the stalk of these plants were dissected and number of larvae, larval mines and parasitized larvae per plant were recorded. The results showed that, 14 species of natural enemies of the European corn borer collected from the experimental field in Moghan region and were identified. The adults of this predator is relatively large bodies. Lacewing *Chrysoperla carnea* predator *Orius niger* and hybrid MV524 among predators on maize European stem borer, respectively frequency more in both years were studied. among European corn borer moth predators such as *Orius niger* highest percentage of corn in both years were studied. Species *Chrysoperla carnea* and *Geocoris uliginosus* and *Nabis pseudoferus* were the highest in terms of frequency, respectively. Also, bee larvae



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and the parasitoid wasp *Trichogramma brassicae* *Habrobracon hebetor* as a eggs parasitoid were collected and identified in the experimental field of corn. In final the results showed that natural enemies of diversity (number of species and the abundance of natural enemies), the hybrid NS704 was higher than the other hybrids.

Key words : Corn stem borer, identification and natural enemies

INTRODUCTION

Corn is a very versatile grain that benefits mankind in many ways. This crop is the most important cereal crop in Iran and with rice and wheat, maize is one of the three most important cereal crops in the world. Maize is high yielding, easy to process, readily digested and cheaper than other cereal crops. It is also a versatile crop, growing across a range of agro ecological zones. Every part of the maize plant has economic value which the grain, leaves, stalk, tassel and cob can all be used to produce a large variety of food and non food production. Corn. Each year, 6 billion bushels of corn are used as feed for cattle, hogs and poultry in the United State. Another 2 billion bushels were exported, which is an integral part of this country's balance are converted to sweeteners, starch, flower cereal, liquor, animal feed, vegetable oil, alcohol for fuel and hundreds of other products (Beyranvand et al, 2013). Forgive to highest seed yield in agriculture addition to both fertilizer and pest management is very important (Shaban, 2013a,b in chickpea; Azimi et al, 2013in wheat and Beyranvand et al, 2013). P

Pests (Lepidoptera: Pyralidae) in maize (*Zea mays* L.) production in Moghan region, Iran. European corn borer larvae cause yield losses of up to 30% in regions with a high natural occurrence of ECB due to feeding and tunneling in plants (Jarvis et al. 1974, Bohn et al. 1998).

Corn borer (ECB) is a common pest of corn in Ohio that may cause economic losses during the growing season. European corn borer infestations differ over time and among geographic regions in the state. Where ECB is active, the development of borers in corn stalks interferes with the flow of nutrients in the host plant, enhances infection by stalk diseases, causes stalk breakage and ear drop, and reduces corn yield. Furthermore it is assumed that ECB damage favors secondary mold infections such as *Fusarium* spp. or *Ustilago maydis*, which may led to additional yield losses and adversely affect the quality of grains (Lew et al. 1991, Munkvold et al. 1997, 1999).

ECB overwinters as late instar or stage larvae in corn stalks. In the early spring, the overwintering larvae pupate and then emerge as moths that prefer to deposit their egg masses on the underside of leaves of mid-whorl stage corn. Each egg mass appears like a small mass of fish scales and may include 15 to 20 eggs. Larvae of the first brood pupate in the stalks and emerge again as adult moths in late July and early August. These adults prefer to deposit their eggs on late-planted corn. The larvae hatching from this generation are referred to as the second brood; they will overwinter as late-instar larvae in corn stubble. Larvae will feed on the surface of the leaves before tunneling into the corn stalk where the predominant feeding occurs. The ECB originated in Europe and expanded to the Middle East, northern Africa, North America, and Central America (Hoffmann und Schmutterer 1999). The Z-race of ECB primarily depends on maize as the host plant and, therefore, developed into an important maize pest since maize production rapidly increased in Central Europe (Langenbruch and Szweczyk 1995). In contrast, the polyphagous E-race attacks a wide range of herbaceous wild and cultivated plant species with stems large enough for larvae to enter, e.g., *Polygonum* spp., *Urtica* spp., and *Solanum tuberosum* L. (Hudon and LeRoux 1986). In Central Europe the ECB completes normally one generation (univoltine) per year. In warmer regions, ECB occurs with two or more generations (multivoltine), depending on the geographic latitude and regional climatic conditions. Moths of ECB occur mid of June until the second half of July and oviposit egg masses onto maize plants in the late whorl stage before anthesis. After 10 to 14 days larvae begin to hatch. First- and second-instar larvae feed initially on leaf tissue



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within the whorls and then attack the enclosed tassels, feeding on the developing anthers. Later instars prefer pollen that accumulate in the leaf axils, attack the ears and shanks before boring into the stem of the plant. The adult larvae feed extensively in the stalks moving downwards to the bottom of the stalk or inside the ear to diapause (Hoffmann and Schmutterer 1999). Yield losses due to corn borer can be attributed to a combination of stalk injury by first- or second-brood larvae, ear drop due to second-brood injury to shanks and ears, and enhancement of stalk rot due to microbial infection of injured stalks. In general, if one larva tunnels and completes its development per stalk, the assumption is that a 5% yield reduction may be expected. The severity of ear drop and stalk breakage depends on the incidence and location of borer cavities and environmental conditions favoring plant infections by microbial agents.

However, this experiment was laid out in order to Identification of natural enemies of maize stem borer of *Ostrinia nubilalis* (Hubner) on four corn hybrids in Moghan region.

MATERIALS AND METHODS

This experiment entitled the effect of density of eggs and larvae of *O.nubilalis* and percentage of eggs and larvae parasitism were laid out on four corn hybrids NS704 (commercial cultivar), NS770, NS640 and MV524 (cultivars with high yield) under temperate condition in station of agricultural farm in Ardabil province (Moghan station), Iran during 2011 and 2012. The experiment was conducted in a completely randomized block design with four replications. The predators and parasitoids of the European corn borer were collected and identified on four corn hybrids in an experimental field. The seeds of corn hybrids were planted in an experimental field, approximately 700 m². Samplings were conducted from the V6 (six leaf collar) until the R3 (milk) growth stages of corn.

For identification of natural enemies of maize stem borer species in samples collected from the four hybrid maize field study was conducted the following experiment:

In primary predators on plants of each maize hybrids studied the details of the date of biopsy, called hybrid corn and corn growth stages were collected. If predators or nymph stage larvae were collected in the laboratory corn borer larval stages of the predators were fed up with the hunt to become the adult stage. After the emergence of adult predators were attempting to identify the species.

For determine the frequency of such natural enemies samples from the four corn hybrids tested in each of the developmental stages of stem elongation with six leaves, the stem with eight leaves, tassel emergence, emerging within the corn silk on the ear, and the dough were . At each turn randomly sampled 20 plants for each hybrid were studied. The number of each species of natural enemies in the samples collected (with notes of corn hybrid characteristics such as name, date of sampling, corn growth stage) were counted.

RESULTS AND DISCUSSION

Some of natural enemies

In this study, 14 species of natural enemies of the European corn borer collected from the experimental field in Moghan region and were identified (table1):

***Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae)**

The adults of this predator is relatively large body length of 5/5 to 8 mm. The front rim has a narrow strip of black and white colors, the pollen also is a black and white spots on the margin front, it is shown. Antennae black at the





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base and bottom sections are tan, its posterior margin is arched, red or orange beetles, absolutely convex edges are smooth and rounded bottom. The underside of the body and legs are black and white and covered with fine hairs. Base of the nail is the shelf (Casey et al, 1925) (fig 1).

Hippodamia convergens Guérin-Ménéville (Coleoptera: Coccinellidae)

The adults of this predator semi-elliptical body and a length of 3 to 5/3 mm. The pollen is white with large black spots that have arisen from the fusion of smaller spots covered (fig2) (Frank and Slosser, 1996 and Casey et al, 1925).

Exocomus nigromaculata Goeze (Coleoptera: Coccinellidae)

The adult beetles are a half-circle shape of the body and its length is 3 to 4.5 mm, the black disk is transparent and brownish yellow rear side corners of in males the front of the head is yellow, yellow is the first paragraph of the abdomen and legs, paramere of Adagvs too long has long shaggy hair (fig 3) (Casey et al, 1925).

Frequency of the European corn borer moth predators

Lacewing *Chrysoperla carnea* predator *Orius niger* and hybrid MV524 among predators on maize European stem borer, respectively frequency more in both years were studied (fig 5 and 6).

The percentage of predators *O. niger*, *C. carnea* and *Hippodamia convergens* on hybrid NS640 were more compared with other predators in both years of the study (fig 7 and 8).

In this study, 12 species of prey belonging to the family Miridae, Geocoridae, Anthocoridae, Nabidae, Chrysopidae, Coccinellidae and Hemerobidae corn experimental farm located in regions of Moghan were collected and identified. Our results showed that among European corn borer moth predators such as *Orius niger* highest percentage of corn in both years were studied. Species *Chrysoperla carnea* and *Geocoris uliginosus* and *Nabis pseudoferus* were the highest in terms of frequency, respectively. Also, bee larvae and the parasitoid wasp *Trichogramma brassicae* *Habrobracon hebetor* as a parasitoid eggs were collected and identified in the experimental field of corn. The predators and parasites on all four hybrids of maize were studied. (Coll, and BotterII, 1991; Godfreyet al, 1991 and Lewis et al, 2005).

The egg parasitoid *Trichogramma* spp. And parasitoid larvae *Habrobracon hebetor* (Say), ladybird species belonging to the family Coccinellinae, scenes predator *Orius* spp. And *Macrolophus* spp. And lacewing larvae of *Crysoperla* spp. As natural enemies of the pest have been reported. Evanse (2009) showed that among the Coccinellidae family of beetles, European corn borer moth predators that feed on the eggs of the pest, and about 10 to 20 percent of the eggs to destroy the pest. Eigenbrode et al (1996) High efficiency predator *O. insidiosus* to control larvae of *Plutella xylostella* (L.) on cabbage cultivars compared with cultivars of cabbage leaf with clear wax with a matte wax on the leaf surface to the greater mobility of the predator on the leaves cultivars were compared with translucent wax. Scot Brown et al (1999) reported that the presence of hairs on the leaves of *Dombeya acutangula* Cav. *O. laevigatus* fed predator of thrips have a negative effect. Atakan and Rosen (1996) and Dissevelt et al (1995) reported that *Orius* ages to plant more flowers attract and feed pollen in the absence of prey.

In this study, the four hybrid corn study halls lowest larval density and the lowest number of larvae per plant were observed on hybrids NS704. In addition, the minimum tunnel length on a hybrid larvae were NS704. The results show that the hybrid NS704 compared with three-dimensional hybrid utility less than the study of European corn borer is the larval feeding. In addition, the percentage of parasitized eggs and larvae from the hybrid NS704 further three-dimensional hybrid was studied. This result is likely due to the high diversity of natural enemies have been





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NS704 Hybrid. In addition, the utility is less than the NS704 Hybrid corn borer larval feeding causes the larvae feed on the plant, seeking suitable locations. This behavior causes the displacement of the hybrid larval parasitoid *H. hebetor* are more exposed and thus the hybrid larval parasitism was higher than the other hybrids.

In final the results showed that Natural enemies of diversity (number of species and the abundance of natural enemies), the hybrid NS704 was higher than the other hybrids. Against the European corn borer larval population density on the hybrid was significantly lower than other hybrids. These results suggest that NS704 Hybrid has the potential for application in integrated pest management programs in the European corn stem borer.

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Table 1. Natural enemies of the European corn borer moth species collected from the experimental field of corn in 2011 and 2012 in Moghan region

Order	family	Genus and species
Coleoptera	Coccinellidae	Coccinella septempunctata Linnaeus
		Hippodamia convergens Guérin-Ménéville
		Exocomus nigromaculata Goeze
		Propylaea 14 punctata Linnaeus
		Scymnus syriacus Marseul
Hemiptera	Geocoridae	Geocoris uliginosus Say
	Nabidae	Nabis pseudoferus Remane
	Anthocoridae	Orius niger Wolff
	Miridae	Macrolophus caliginosus Wagner
Neuroptera	Chrysopidae	Chrysoperla carnea Stephens
	Hemerobidae	Hemerobius stigma Stephens
	Braconidae	Habrobracon hebetor Say
Hymenoptera	Trichogrammatidae	Trichogramma brassicae Bezdenko



Figure 1. Adult *Coccinella septempunctata*



Figure 2. Adult *Hippodamia convergens*





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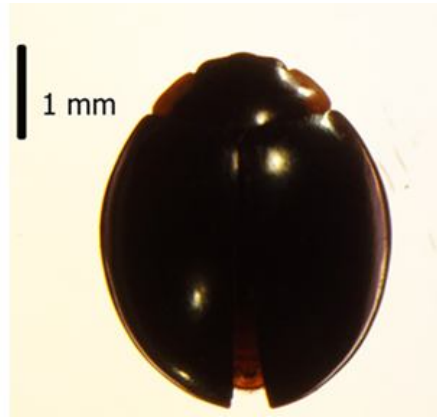


Figure 3. Adult Exocomus nigromaculata

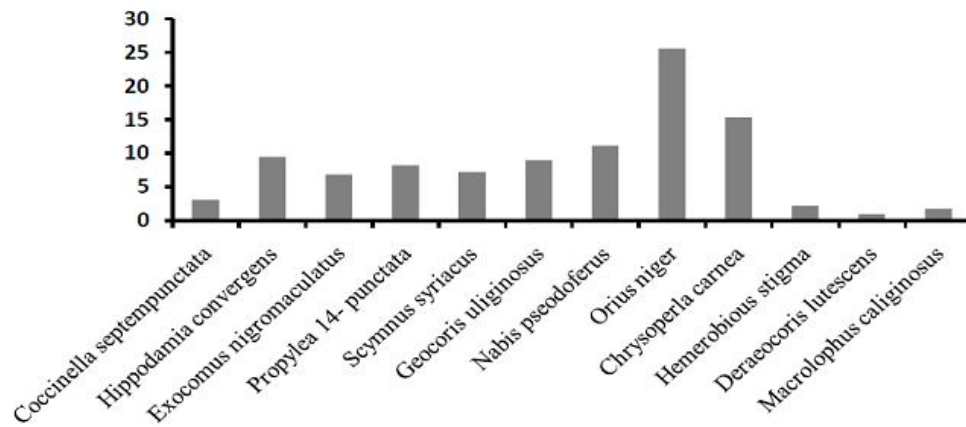


Figure 5. The percentage of European corn borer moth predators on hybrid MV524 in 2011.

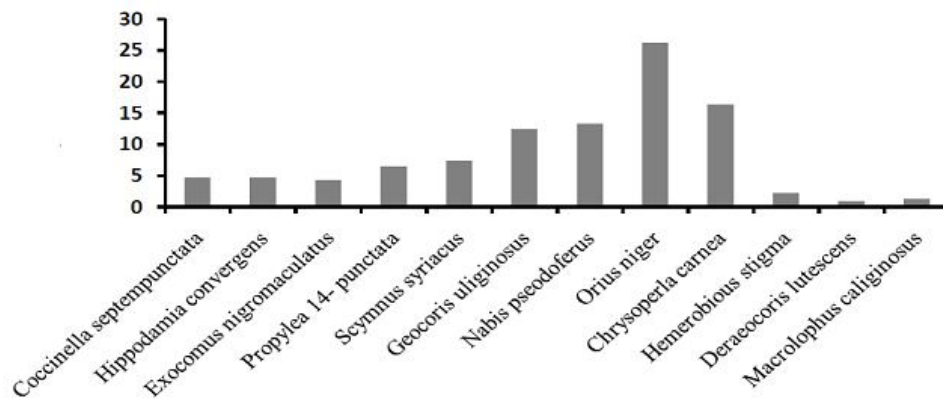


Figure 6. The percentage of European corn borer moth predators on hybrid MV524 in 2012.





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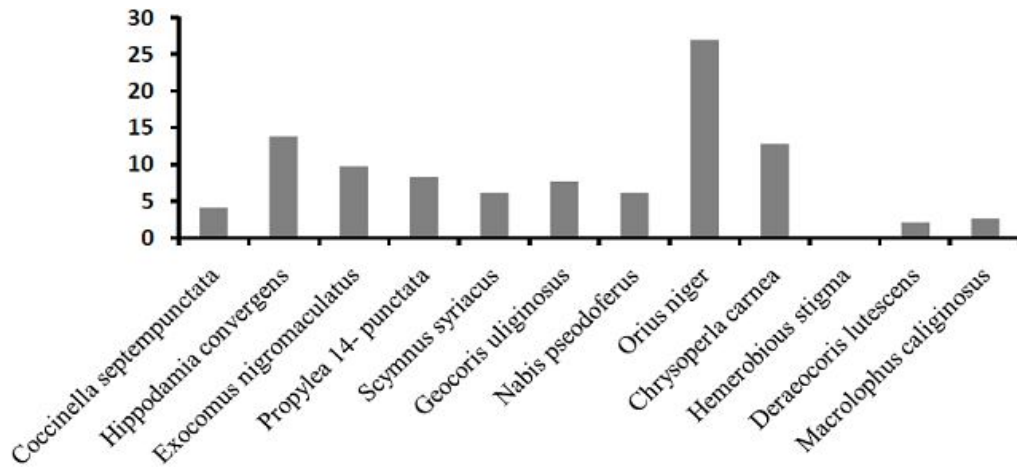


Figure 7. The percentage of European corn borer moth predators on hybrid NS640 in 2011.

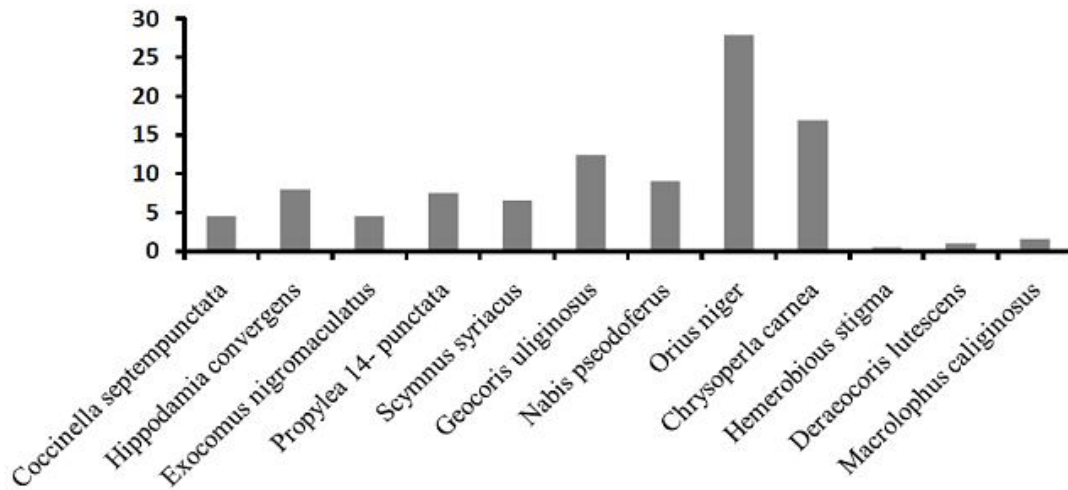


Figure 8. The percentage of European corn borer moth predators on hybrid NS640 in 2012.





RESEARCH ARTICLE

Prediction of Mackerel Landings Using MODIS Chlorophyll-A, Pathfinder SST and Seawifs PAR

N.Madhavan^{1*}, D. Thirumalai Vasan², K. Ajith Joseph³ and K.Sravani¹

¹ College of Fishery Science, SVVU, Muthukur-524344, Andhra Pradesh, India.

² Institute of Remote Sensing, Anna University, Guindy, Chennai- 600025, TamilNadu, India.

³ Nansen Environmental Research Center (INDIA), Sreekanath Road, Ravipuram, Kochi, 682016, India.

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*Address for correspondence

Dr. N. Madhavan

Associate Professor, College of Fishery Science,
SVVU, Muthukur-524344,
Andhra Pradesh, India.

E-mail: madhav_irs@yahoo.com



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ABSTRACT

A total of Fourteen Artificial Neural Network (ANN) models were developed to predict 12 month ahead monthly Mackerel landings was analyzed for Bay of Bengal, Tamil Nadu (Nagapattinam dt) coast, India, previous 60 months as inputs to the models. This Neural Network Models developed with Time Series of ocean colour parameters such as Chlorophyll-a (CHL), Sea Surface Temperature (SST) and Photosynthetically Active Radiation (PAR) as input in Time Series as dependent variable for the target of Mackerel catch time series in the study area as Seasonal (12 lag) and Non-Seasonal models for this study. The output from seasonal and Non-Seasonal models were compared and the seasonal models were out performed Non-Seasonal models in prediction. The model STS_MO_MAC_CP_S (R^2 between the predicted and observed landings is about 0.8818) performed well when compare to other Seasonal, Non-Seasonal Univariate and Multivariate Neural Network models. This study demonstrates that the ocean colour parameters Chlorophyll-a (Chl-a), Sea Surface Temperature (SST) and Photosynthetically Active Radiation can be used in the study area on Mackerel landing prediction. In general, seasonal ANN exhibits good performance in prediction of Mackerel catch landings when compare to Non-Seasonal ANN architecture.

Key words: Chlorophyll-a, Sea Surface Temperature, Photosynthetically Active Radiation. and Neural Network.



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INTRODUCTION

Ocean colour remote sensing is applied more and more widely in the water quality monitoring of oceanic, Case 1 and Case 2 water bodies, because it has many advantages, such as, wide range synchronization and low cost for data collection. Satellite remote sensing of ocean colour information on chlorophyll concentration, SST, wind speed and Sea Surface Height (SSH) gives better understanding about Oceanographic processes such as, currents, ocean fronts, rings, eddies and coastal upwelling. In this context, the first Space borne ocean-colour sensor Coastal Zone Colour Sensor (CZCS) was launched in the year 1978, which provided data until 1986. This was followed by several new sensors such as, SeaWiFS (1997), MODIS in 1999 and 2002 and recently by OCEANSAT-2 OCM on Sep 23 2009, is added to the list. In this study, three satellite derived ocean environmental parameters (CHL), (SST) and (PAR) are considered as input variable for Neural Network Modelling (NNM) to predict the monthly landings of Mackerel at Nagapatnam District, Tamil Nadu, India.

MATERIALS AND METHODS

Study area

The study area Nagapatnam coastal district (White in color) in Tamil Nadu, eastern part in Bay of Bengal covering a coast line length of 190 km. Figure.01 explains the schematic representation of Nagapatnam district falls in the latitudinal and longitudinal extensions between 10°46'1.2"N and 79°49'58.8"E on the Eastern part of Bay of Bengal. The movement of fishermen and the seaside limit (1000 m isobaths) for the extraction of Sea Surface Temperature, Chlorophyll-a and Photosynthetically Active Radiation in bay of Bengal. The minimum and maximum temperature are 20 °C and 34 °C. This district comprises of five coastal taluks that is Nagapattinam, Sirkazhi, Tarangampadi, Mayiladuturai and Vedaranniyam.

Artificial Neural Networks

There are so many different ways to forecast nonlinear phenomena, among them Neural Networks is the best efficient method to get forecast information from a nonlinear time series (Lin sun et.al 2009). There by the applications of neural networks to fish landing time series forecast have become very popular over the last few years, since most of the landings time series are in nonlinear pattern. Neural networks are simple nonlinear computing units and just imitating human neural system has an input layer, a hidden layer and an output layer. Layers in between input and output layers are generally called as hidden layers and commonly referred as neurons. When data is loaded in the ANN (Artificial Neural Network), it must be preprocessed from its numeric range into the numeric range that the ANN can deal with efficiently to improve the efficiency of the learning results (Kim and Lee, 2004).

Fishery data

For the preparation of Time Series (TS) data interms of Indian Mackerel (*Rastrelliger kanagurtha*), monthly landing details were obtained from the Central Marine Fisheries Research Institute (CMFRI) Cochin, database. The period considered for Mackerel TS is from 2005 to 2010, out of which the CMFRI data pertains to the period 2005-2009. For validation purpose, fish catch data for the year 2010 from fish landing centers were collected. Standardization of commercial catches through Catch Per Unit Effort (CPUE) could provide a powerful method for estimating trends in the stock abundance. Unfortunately, there were many aspects of fishermen's behaviour that will cause CPUE to be not proportional to abundance even on a very small spatial scale (Hilborn and Walters 1992). So, for this study, Mackerel landing details are considered instead of CPUE for the preparation of fish landing TS. The data were



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collected by qualified and well-trained technical staff of CMFRI by following stratified multi-stage random sampling technique, in which the Mackerel landings were recorded by covering landing centers along the Nagapattinam coast. The catch data for the year 2010 (on monthly basis) were collected in entire Nagapattinam coastal area from all forty four landing centers physically with the help of Department of Fisheries, Officials. A team of fourteen local fishermen were engaged to cover the entire 44 fish landing centers for the collection of fish catch data. This data is used for testing and validating NNM predicted fish catch.

Satellite data.

Chlorophyll-a concentration (CHL)

The primary function of Chlorophyll-a is photosynthesis of marine algae in the ocean, which is the main food for Mackerel larvae and that availability of food during the critical developmental period of Mackerel larvae determines the year class of Mackerel population is important for Mackerel availability in Bay of Bengal. So Chlorophyll-a in Bay of Bengal is considered in the prediction models in this study. MODIS level 3 standard binned images archived by the Ocean Biology Processing Group (OBPG) were used to estimate sea-surface chlorophyll-a concentrations. These data were obtained from the <http://oceancolor.gsfc.nasa.gov/cgi/l3> . for this study Global Area Coverage (GAC) monthly composite MODIS images with a spatial resolution of about 4.5 km for the period from January 2005 to December 2010.

Sea Surface Temperature (SST)

In Mackerel life cycle Sea Surface Temperature is an important factor which leads the Mackerel activity levels to increase or decrease, makes Mackerels move into certain areas, and influences feeding and reproductive activity. Temperature data are of interest to those who wish to catch fish or study them. Since the Mackerel is a tropical fish we need to understand how temperature affects fish behavior. So Sea Surface Temperature also considered as one of the main environmental factor in the Mackerel landing prediction which is normally preferring temperature range of 27° to 29°C (Chidambaram, 1950). The NOAA pathfinder data presented in ftp://podaac.jpl.nasa.gov/allData/avhrr/L3/pathfinder_v5/monthly/ data were used to study SST for the same period having the same spatial resolution along with chlorophyll data.

Photosynthetically Active Radiation (PAR).

Photosynthetically Active Radiation (PAR) is the amount of light available for photosynthesis, which is defined as the quantum energy flux from the Sun light in the 400 to 700 nanometer wavelength range. Since the Mackerels are herbivores Photosynthetically Active Radiation also considered as one of the biophysical parameter. PAR changes seasonally and varies depending on the latitude and time of day. This data set consists of algorithm estimates of global Photosynthetically Active Radiation (PAR) reaching the surface obtained by the Sea-viewing Wide Field-of-view Sensor (SeaWiFS), in orbit on the OrbView-2 (formerly SeaStar) platform. SeaWiFS data products are processed and distributed by the Ocean Biology Processing Group (OBPG). For this study SeaWiFS Level 3 monthly Binned data files are downloaded from the FTP site at <http://oceancolor.gsfc.nasa.gov/cgi/l3> having 9 km spatial resolution.

Image processing

Monthly mean CHL, SST and PAR images for the entire period were downloaded, processed and extracted in BEAM software developed by European Space Agency(ESA). BEAM is an open-source toolbox and development platform for viewing, analyzing and processing of remote sensing raster data. BEAM supports a number of raster data formats such as GeoTIFF, HDF and NetCDF as well as data formats of other EO sensors such as Moderate Resolution





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Imaging Spectroradiometer (MODIS) and Advanced Very High Resolution Radiometer (AVHRR). The remote sensing data extracted for this study was in both NetCDF and HDF format, which is supported by BEAM software. A total of 216 images were downloaded, processed and spatially averaged as point data for each polygon for this TS preparation. A 1000 meter isobath of Tamil Nadu region in Bay of Bengal was digitized in ARC GIS environment. This isobath shape file again bifurcated into district polygons of all Tamil Nadu coast to extract monthly mean information for CHL, SST and PAR. These polygons are imported into BEAM software along with monthly mean images of CHL, SST and PAR parameter to get the month wise time series of all 12 polygons (coastal districts) for six years from 2005 to 2010.

METHODOLOGY

The time series of Nagapatnam district Mackerels monthly landings were normalized from zero to one by simply dividing the real value by the maximum of the appropriate set because of their nonlinearity. The time series belongs to CHL, SST and PAR is kept as such, since they are having only seasonal influences on it. The in-situ Mackerel landing time series collected for the year 2010 January to December in all the landing centers of Nagapatnam district. The spatially averaged CHL, SST and PAR value extracted from images of Thiruvallur District to Ramanathapuram District. The Mackerel landings time series modeled in Matlab (R2012a) Neural Networks to get the prediction values for the year 2010 and compared with the insitu catch data. In this NN function the CHL, SST and PAR parameter up to 2005 - 2009 as input against the year 2010 as target to model to predict the Mackerel monthly catch for the year 2010 to Nagapatnam area.

METHODS OF EVALUATION.

Several measures of accuracy were calculated in the calibration between model output and observed value. A measure of correlation between the observations and the predictions is the coefficient of correlation (R). The proportion of the total variance in the observed data that can be explained by the model was described by the coefficient of determination (R²). The estimators to quantify the errors in the same units of the variance were the square Root of the Mean Square Error (RMSE), and the Mean Absolute Error (MAE). On the other hand other measures of variance were the Coefficient of Efficiency (E₂) (Nash and Sutcliffe, 1970; Kitanidis and Bras, 1980), the Average Relative Variance (ARV) (Grin o. R. 1992), and the percent Standard Error of Prediction (SEP) (Ventura et al., 1995) also analyzed for sensitivity analysis in this study. The E₂ and AVR were used to see how the models explain the total variance of the data and represent the proportion of variation of the observed data considered for Mackerel forecasting modeling. The SEP allows the comparison of the forecast from different models and different problems because of its dimensionless. For a perfect performance, the values of R² and E₂ should be close to one and these of SEP and ARV close to zero. The optimal model is selected when RMSE and MAE are minimized. The above estimators are given by:

$$R = \frac{n \sum Y_i \hat{Y}_i - (\sum Y_i)(\sum \hat{Y}_i)}{\sqrt{n(\sum Y_i^2) - (\sum Y_i)^2} \sqrt{n(\sum \hat{Y}_i^2) - (\sum \hat{Y}_i)^2}} \quad (1)$$

$$E = 1.0 - \frac{\sum_i^n |Y_i - \hat{Y}_i|^2}{\sum_i^n |Y_i - \bar{Y}_i|^2} \quad (2)$$





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$$ARV = 1.0 - E^2 \tag{3}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n |Y_i - \hat{Y}_i|^2}{n}} \tag{4}$$

$$MAE = \frac{\sum_{i=1}^n |Y_i - \hat{Y}_i|}{n} \tag{5}$$

$$MAPE = \frac{100}{n} \times \sum_{i=1}^n \left| \frac{Y_i - \hat{Y}_i}{Y_i} \right| \tag{6}$$

$$SEP = \frac{100}{\bar{Y}_i} RMSE \tag{7}$$

Where Y_i is the observed value, \hat{Y}_i is the forecasted value to Y_i , and n is the number of the observations of the validation set. \bar{Y}_i is average mean value of the target.

RESULTS AND DISCUSSION

The uncertainty in traditional fishery model’s effectiveness is mainly because of the variability increment with time and ecosystem types on fishery landings (Stergiou (1991, 1996) and inaccuracies in model input data sets during data collection and time-series gaps also leads discrepancies in various model predictions. Because of all the above reasons, predictions which may influences fisheries management plans have shifted from a Univariate single-species approach to an ecosystem-based multivariate approach (Stergiou, 2002). Based on the above reasons, in this study a variety of environmental parameters derived from satellite remote sensing have considered for the prediction of Mackerel fish landings in Nagapatnam district. In this paper, time series data, (2005 to 2010) of SST, CHL and PAR for Six years is considered as environmental input variables for NNM to predict the Mackerel landing in the study area

From Figure 2.(a) it is observed that March to June, when hot winds prevail, the SST will be high. The occasional showers of the South-west monsoon and subsequent river discharges, cools down the temperature gradually. Thereafter with setting of the North-east monsoon, the temperature steadily falls till December, when it reaches the minimum. The atmosphere continues to be cool till the weather becomes moderately warm during day though still cool at nights and then in the later part of March the hot season commences. The monthly temperature ranges between 25° C to 32° C. the mean monthly temperature is 27.7° C. From Figure 2.(b) it is observed that June to July when SST is low the CHL will be high. An inverse relationship between CHL concentration and SST was observed





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from Figure 2.(a) and 2.(b). The monthly CHL ranges between 0.32 to 0.93 Mg/m³. The mean annual CHL concentration is 0.58 Mg/m³. From the Figure 2. (c) it is observed that the PAR distribution in the study area is showing high, when SST is high and CHL is low. Hence, PAR is experiencing an inverse relationship with CHL and a linear relationship with SST. The monthly PAR ranges between 20.48 in December to 54.72 Ein/M²/day in April. The mean annual PAR is 44.9 Ein/M²/day. Figure. 3. shows that landings of Mackerel is high during the period May-July. The Government of Tamil Nadu has declared that the month of May (i.e. actually from April 15th to May 30th) is a closed season for fishing. However, since non-mechanised vessels and mechanised vessel (<10hp) are allowed during the period for fishing. Observations on Mackerel made at Mandapam on the south east coast of India indicated the possibility of two spawning periods, one during October-November and the other major spawning during May-June (CMFRI Annual Rept., 1957). The inference drawn by Bhimachar and George (1952) that food could be a major factor governing these migrations is contended by Sekharan (1965). He also felt that without studying the plankton available in the offshore waters the shoreward migration should not be linked with the food factor.

Table 1. shows the sensitivity analysis of MODIS CHL's performance over the Mackerel landings through NNM. In this modeling, Mackerel has been targeted by all the time series. Figures 4. and 5. a and b shows the STS MODIS Mackerel Neural Network model predictions in the study area in both Seasonal and Non-Seasonal pattern. According to Table. 1. the best Neural Network model among Non Seasonal and Seasonal model is STS_MO_MAC_CP_S, which gives the model accuracy of R²=0.7776, % SEP=46.4266, E=0.5311 and MAPE = 25.1544. The NN structure for the rank best Neural Network is 2:05:01 with 400 epochs. From the result it is obvious that independent variable CHL and its combinations are having more correlation next to STS_MO_MAC_CP_S and the model STS_MO_MAC_CHL_S is ranked 2nd. the model STS_MO_MAC_SC_S stood 3rd and finally the fourth rank is achieved by the model STS_MO_MAC_CSP_S.

Among Non-Seasonal Neural Network modelling the model STS_MO_MAC_SP_NS stood 8th in the rank in the entire fourteen models, and stood first among Non-Seasonal NNM. The structure for the best NS Neural Network is 2:07:01 with 3000 epochs. The regression performance of all models are higher than 0.6 is found in all the present Seasonal Model. But, some models, the regression performances are not achieved up to the level of 0.6.

STS (Modis) Mackerel Non Seasonal Models

The Figure 4.0 explains the prediction performances among Non-Seasonal STS Mackerel NNM. From the table Table 1.0 the best STS MODIS Mackerel for Non-Seasonal model is STS_MO_MAC_SP_NS which is ranked first among Non-Seasonal with a sensitivity result of R²= 0.2777, % SEP= 95.4423, E= -0.9815 and MAPE = 66.2029 %. In this STS MODIS NN modelling the CHL and its combinations are performed poorly and shifted from positive correlation to negative correlation. Only the models STS_MO_MAC_PAR_NS and STS_MO_MAC_SP_NS gives the positive correlation in this Modelling. From the Figure 4. It is observed that all prediction values are under - estimated except the model STS_MO_MAC_CP_NS, where the month May and December are overestimated. Further the model STS_MO_MAC_SST_NS, overestimate November month observed value.

The low profile landing months are effectively predicted particularly in the months of November, December and May. A high amount of variation observed in the months of January, June and October. Almost all the iterations are under - estimated the above months in prediction.

STS (Modis) Mackerel Seasonal Models

Figure 5. a and b shows the observed vs prediction graphs for seasonal Mackerel NNM for MODIS CHL. All seasonal NNM (except STS_MO_MAC_SP_S) are correlated well with Mackerel landings with a R² greater than 0.60. Further an increased R² in MODIS CHL and its combination NNM prediction level is observed. In MODIS



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CHL almost all the NNM involves in over estimation in the month of June, whereas the month March was underestimated by all the individual models and combination NNM predictions. The model STS_MO_MAC_CP_S perfectly predicts the winter season months (September, November and December). Other models having variations from 60-100% over estimation in September month. The months August and October was almost predicted exactly by all the seasonal models.

The best performance observed in STS MODIS Mackerel seasonal model is STS_MO_MAC_CP_S with a prediction results of $R^2=0.7776$, % SEP=46.4266, E=0.5311 and MAPE=24.1544. Further, in Mackerel STS model analysis the model STS_MER_MAC_CHL_S captured 4th rank in overall performance, but here the model STS_MO_MAC_CHL_S performed next to STS_MO_MAC_CP_S and captured 2nd place. Based on which, it is inferred that the MODIS CHL gives better correlation results with CHL for seasonal Mackerel Neural Network Model in the study area. The combined performance of all seasonal predictions are shown in Figure . 5. a (H) for comparison purpose.

CONCLUSION

In this study the seasonal Neural Network models as a whole is giving good correlation and stood first seven positions out of fourteen models indicates that the seasonal models performed well when compare to Non-Seasonal models. The results from STS_MO_MAC_CP_S on the Mackerel fish landings have shown 88% correlation and a minimum MAE of 0.0029 between observed and estimated Mackerel landings in Nagapatnam district. The models STS_MO_MAC_CHL_S, STS_MO_MAC_SC_S, STS_MO_MAC_CSP_S are stood 2nd, 3rd and 4th having good correlation next to STS_MO_MAC_CP_S. It is concluded that all the Chlorophyll-a and its combination models are performed and occupy the first four position and this indicate that Mackerels are highly associated with Chlorophyll when compare to the Sea Surface Temperature. Further, the results showed that high prediction capacity of seasonal NNM with satellite environmental variables, even though the fish catch landings are having strong nonlinear relationship.

This gives the Mackerels in the study area are closed associated with Chlorophyll-a not with temperature in Bay of Bengal. However more research required to get deep understanding of the extent and nature of the relationships between environmental variables and Mackerel landings in the study area. With respect to the performance on amount (high and low) of landing and its prediction, seasonal Multivariate models giving high correlation and less variance. Winter months are modeled better in both Non-Seasonal and Seasonal models. The Non-Seasonal model trying to performs well only when the landings are high and perform well in the low landing winter seasons. To understand the complexities and seasonal nonlinearities, further studies are requires on the nature of uncertainty associated with the food and feeding habits of Mackerel, and the physiological changes which influence the ocean environment.

ACKNOWLEDGEMENTS

The authors are highly thankful to NERSC, Bergen, Norway and Cochin, India for providing opportunity to work in their laboratory to design the model and also to provide the time series landings data from CMFRI through NERSI India. Special thanks to the Mr. A. Elango, Deputy Director Of Fisheries, Tamil Nadu Fisheries Department, Chennai, and his team, for their help in collection of in-situ Mackerel landing data in Nagapatnam district.





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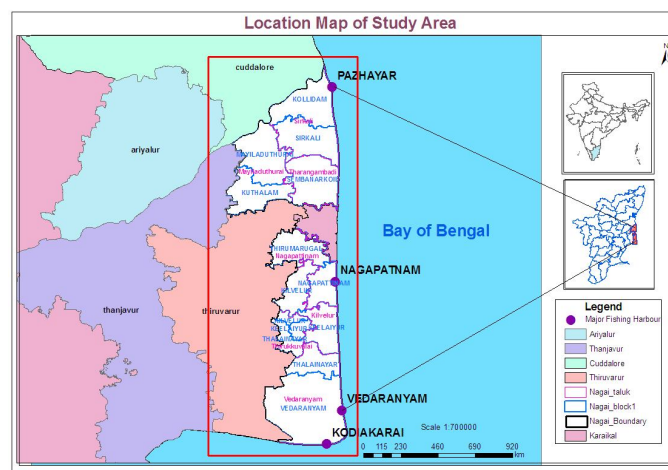


Figure 1. Study Area Details Of Nagapatnam District in Bay of Bengal





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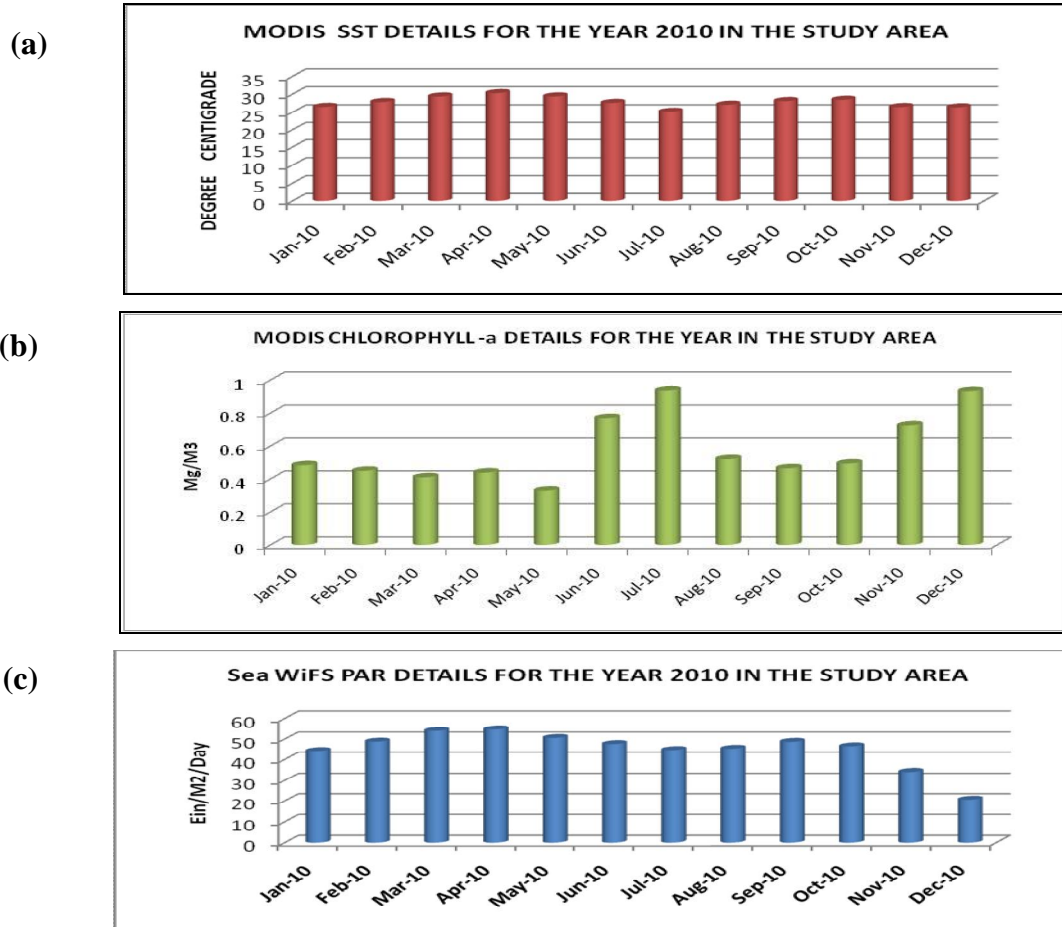


Figure 2. Monthly Mean value of SST (a), CHL (b) and PAR (c) in the study area

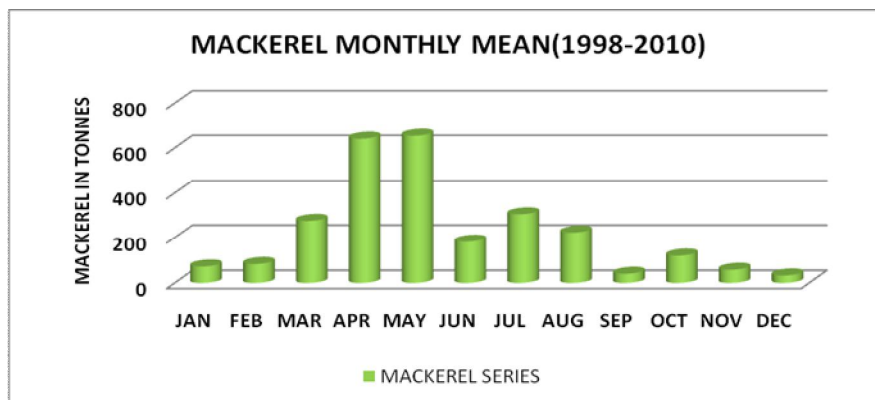
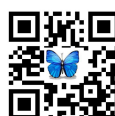


Figure 3. Monthly average variability (1998-2010) of Mackerel landings.





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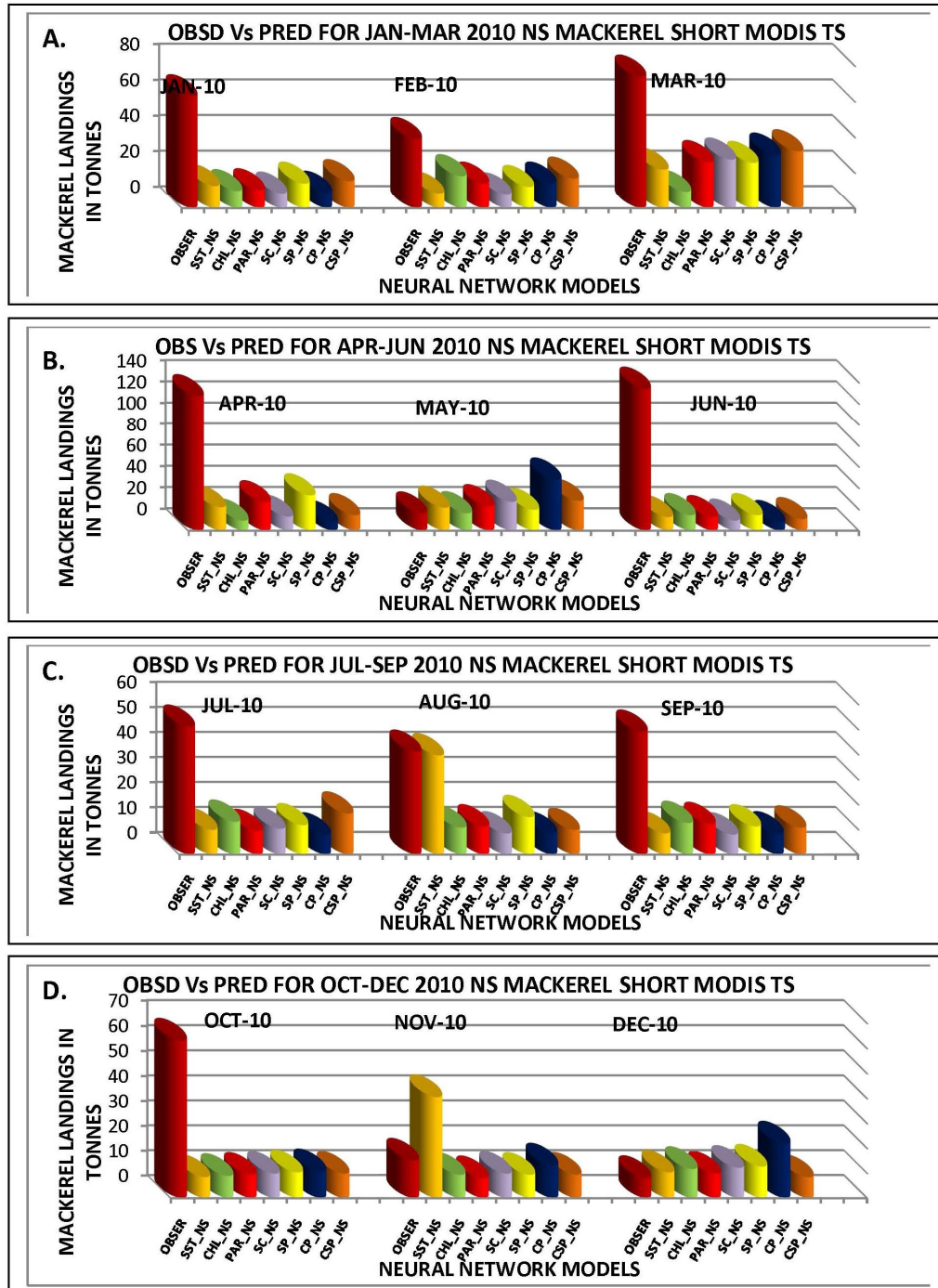
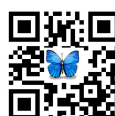


Figure 4. 0STS (Modis) Mackerel Non Seasonal fit for all monthly combinations





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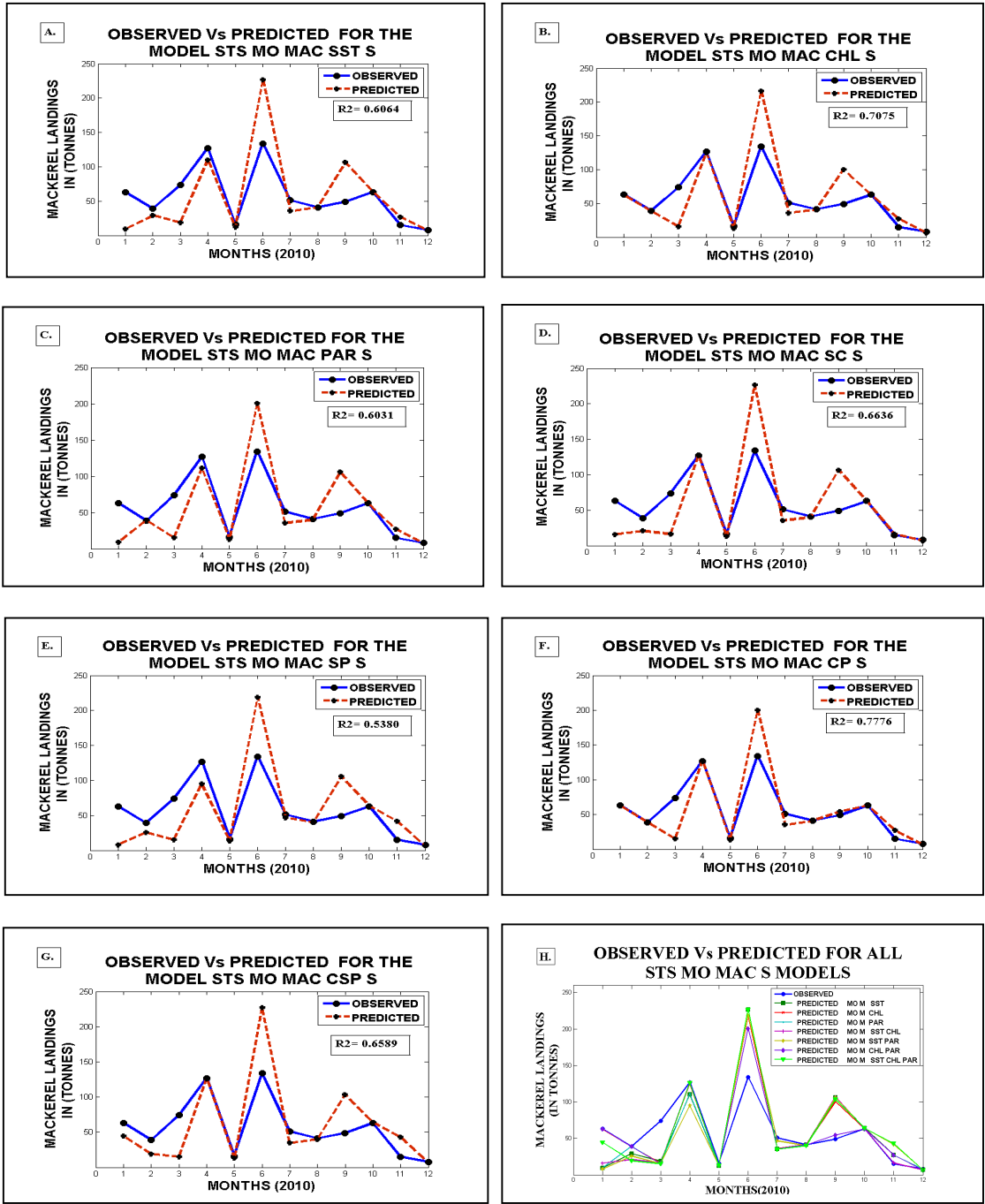


Figure 5.(a) STS (Modis) Mackerel Seasonal fit for all model combinations





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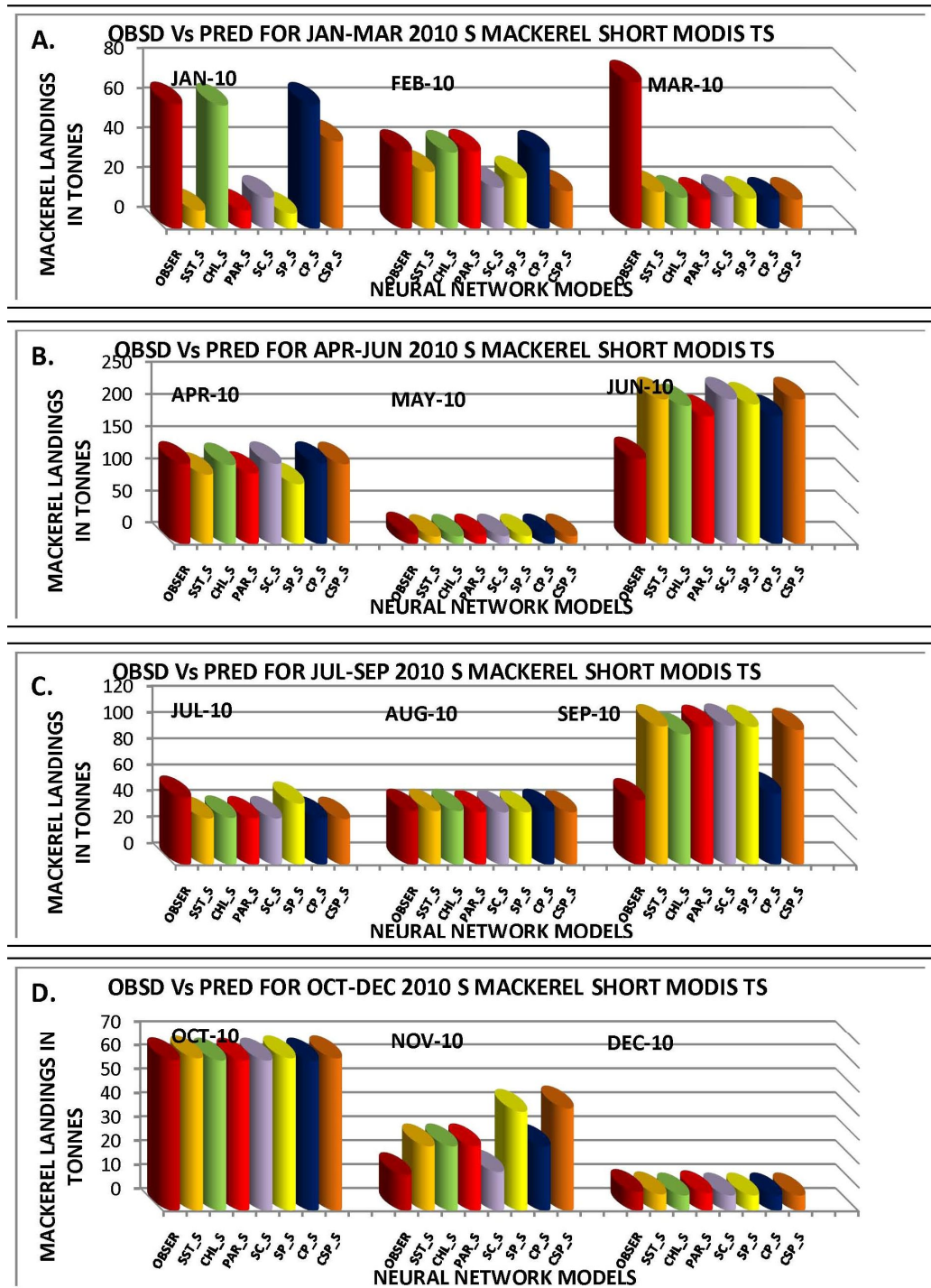
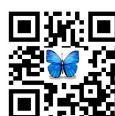


Figure 5.(b) STS (Modis) Mackerel Seasonal fit for all monthly combinations





RESEARCH ARTICLE

Evaluation of Acute Lymphoblastic Leukemia Using Cytochemical Staining

Samanaiubi, Arash Chaichi Nosrati* and Leila Modiri

¹Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University (IAU), Lahijan, Iran.

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***Address for correspondence**

Arash Chaichi Nosrati,
Department of Microbiology, Faculty of Basic Sciences,
Lahijan Branch, Islamic Azad University (IAU),
Lahijan, Iran.
Mobile: +98 912 567 2867
E-mail: Mycotoximune_achn@yahoo.com,



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ABSTRACT

Lymphoblastic leukemia based on cell morphology dominant coloring Cytochemical into three main groups: 1L, 2L, 3L classify, Although the clinical value of the expected lifespan in adults is unknown, but in terms of prognosis and clinical course and response to treatment methods in children is important. 109 peripheral blood samples from patients suspected of Razi Hospital Hematology Center was developed leukemia, and follows Cytochemical staining was performed on each sample, 19 samples by alpha-naphthalen propanoic acetate esterase staining positive 15/78 percent and 84/21 percent were negative. Of the 12 samples analyzed by acid phosphatase staining was found that 25% of the samples were positive and 75% negative, also the 25 samples that were analyzed by alkaline phosphatase staining, 100% of the negative and none of the samples were positive, and 34 samples were examined by staining Periodic acid shifts 97/55% of the negative samples and only 2/94 percent positive, and from 12 samples by staining was evaluated Naphthile AS-D 16/66% positive and 83/33% of the samples were negative. The diagnosis of leukemia, the disease situation and select pin for the treatment of all cases with morphological characteristics of the cells was not possible, therefore, to reach the correct diagnosis Cytochemical tests and immune cell phenotype and cytogenetic studies are needed.

Key words: Acute and Chronic Lymphoid Leukemia, Cytochemical, Prognosis



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INTRODUCTION

Hematologic malignancies involving bone marrow and lymph node diseases that originate, early disorders, including leukemia and bone marrow and immunoproliferative diseases (eg Mlyvm sclerosis) and myeloproliferative syndromes (eg, myeloid metaplasia with myelofibrosis and Polycythemia) Yueet al. (2015). How to treat a patient with Polycythemia absolutely must be done through the logic to determine if patients with Polycythemia secondary or primary (Polycythemia Vera),(Shrestha S., 2013). The history and physical examination, the patient may not respond to this question, a man with lung and chest, wheezing, cyanosis and severe chronic obstructive pulmonary disease, barrel that is the speed of a patient HET (Paul Toric) with splenomegaly with Polycythemia Vera is most likely, will be diagnosed respectively(Yamamoto K et al., 2015),(Sharma P et al., 2014).Simple laboratory data in most patients with Polycythemia Vera arehigh, however, most patients with Polycythemia secondary normal. Despite the low level of oxygen saturation, because that Polycythemia is a compensatory mechanism to hypoxemia, therefore, history, physical examination and laboratory investigation, will differentiate between primary and secondary Polycythemia,of course, sometimes the cause is not obvious Polycythemia secondary may need more tests to find the cause is rare.The rare causes include abnormal hemoglobin with increased affinity to oxygen, renal cysts secretion of erythropoietin, Hemangioblastoma brain, and hepatocellular carcinoma,Polycythemia Vera in the normal control of cell production in the bone marrow is removed, although the erythroid involved and this involvement will increase in red cell mass, the increased production of granulocytes and platelets in most cases it can be seen, Polycythemia Vera has some differences with other myeloproliferative clinical symptoms (ie, myelofibrosis with myeloid metaplasia, essential thrombocythemia, chronic myelogenous Vlvsmy), Yueet al.(2015).

MATERIALS AND METHODS

Sterile speculum, Sterile cotton swab,Glass slides,diamond stylus,ethanol,methanol and acetone, absolute homogeneity,toluidine blue staining kit. Alpha-naphthalen propanoic acetate esterase kit, Naphthile AS-D chloroastat esterase,acid phosphatase,PERIODIC-ACID SCHIFF (PAS), all of Manufacture, Sygma- Aldrich company, german product, Olympus company microscope imaging.

MethodsSampling

Knowing documentation Razi Hospital in coordination with the previous sampling methods were referred to the hospital, and from those for blood disorders (leukemia) were referred for biopsy, biopsy was performed. Attending random cluster sampling,Immediately after sampling, and drying the slides fixation solution, all the samples were fixed.

Preparation of samples

Peripheral blood samples were taken every 11 samples were prepared slides, all slides were coded by a diamond pen, the dried slides temporary fixation with a solution that is 1 volume of ethanol, 1 volume of methanol and acetone was prepared 3 shares were fixed, and then slide the boxes we Filing, and the Laboratory of Immunology we moved lahijan Azad University Branch. to investigate the pattern of leukocyte 1 gram of series slides stained with toluidine blue method, according to the morphology of the white blood cells, white blood cells, staining was noted in the population under study is preliminary, so if there is corruption in collecting samples so check white blood cells on is inefficientto investigate the differential diagnosis of adult cells based on pattern recognition Classic 5 cells, white blood cells,Naphthol AS-D chloroastat esterase staining,alpha-naphthalen propanoic acetate esterase,Acid phosphatase,periodic acid-Schiff with the books of commercial construction company Sigma-Aldrich was used.





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Check expand stained

It should all leukocyte cells and adult groups correctly and without error and the ability to separate high from each other be identified. The appearance of Morphological white blood cells in the development of stained, cytochemical colors and patterns observed in Cytochemical staining was compared and a broader pattern of white blood cells to be identified pentavalent. Open the painting that did not feature a suitable extension of the study subjects were excluded.

Morphological changes of cells

All morphological changes of cells, including cell quality, inflammatory changes, stained with toluidine blue and examined.

Check leukocyte cell

Cytochemical staining for evaluation of leukocyte cells are used, and the samples under a saw microscope. Stained with toluidine blue, acid phosphatase, neutrophils are observed. Eosinophils by Periodic acid-Schiff staining, and acid phosphatase, under an optical microscope with a 100× lens were observed. To view basophils with light microscopy of Periodic acid-Schiff staining and toluidine blue test. Mast cells in the toluidine blue and PAS staining visible, Monocytes by alpha-naphthalene propane acetate esterase and Naphthol AS-D chloroacetate esterase staining stained are colors. Lymphocytes by color, alpha-naphthalene propane acetate esterase, Naphthol AS-D chloroacetate, acid phosphatase, are stained. That's all positive and negative results for acute lymphoblastic leukemia as a percentage of results can be expressed.

Photography

Open stained learn ways to take pictures of each of the white blood cells were used. All stained slides were examined and the typical image of each leukocyte cell of any use Olympus optical microscope with conventional CCD magnification of 100X with a calibrated optical 100 and microscope was harvested imaging software was harvested and software database was coded.

Toluidine blue staining

Slides with a solution of 1 volume of ethanol, 1 volume of methanol, and 3 volumes of acetone fixed, after transfer to the laboratory slides ready to put on a tray coloring, and Toluidine blue on the grapefruit throw, after 15 to 20 minutes offer with water slides, after drying the slides can be viewed with the microscope.

Naphthol AS-D chloroacetate esterase staining

In this method, proven solution by mixing 18 ml of citrate solution, and 27 ml of acetone and methanol (5 ml) was built, and the slides were placed in a minute, the solution to be prove, then washed with deionized water, and then a solution of 6/3 Trizymal by dissolving one part of concentrated buffer 6/3 Trizymal to 9 parts of deionized water was prepared, and added to the asalt capsule V fast Corrientes and then 2 ml Naphthol AS-D chloroacetate esterase solution, added and mixed for 30-15 seconds. all slides were prepared solution for 5 min. were washed with deionized water for 3 minutes. Naphthol AS-D chloroacetate solution by dissolving a capsule Naphthol AS-D chloroacetate in 2 ml dimethyl formaldehyde obtained. painting background slides for 5 to 10 minutes in a solution of haematoxylin acid





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and then rinsed with running water and dried in the open air, target cells by light microscopy to identify the specific and measurement were performed.

Alpha-naphthalen propanoic acetate esterase staining

In this method, proven solution by mixing 18ml of citrate solution and 27ml of acetone and methanol (5ml) was built, and the slides were placed in one minute, the solution to be done was to prove, then rinsed with deionized water, then PH=7/6 Trizymal solution, by solving a 7/6 trizymal dense buffer to 9 parts of deionized water was prepared, and a capsule RR salt, and then add 2ml naphthalen propanoic acetate was mixed for 20-15 seconds. All slides were prepared in the solution for 30 minutes and then washed with deionized water for 3 minutes. naphthalen propanoic acetate solution by dissolving a capsule naphthalen propanoic acetate in 2ml of monomethyl ether glycol obtained, painting background slides for 5-10 minutes in a solution of haematoxylin and then washed in running water were dried in the open air. Target cells by light microscopy to identify species and micrometer measurements were examined and photographed.

Acid phosphatase staining

In this method, proven solution by mixing 25ml of citrate, 65ml of acetone, and 8ml of 37% formaldehyde was made, and the slides were immersed for 3 seconds in this solution to be fixed, then washed with deionized water. Then the two tubes 5.0ml Fast Garnet GBC solution and 5.0 ml of sodium nitrite solution was poured and was mixed for 30 seconds. In a large glass jar, 45 ml of deionized water One ml Fast Garnet GBD solution that the previously prepared 5.0 ml Naphthol AS-BI phosphate solution two ml of acetate was added, and the slides were placed for one hour in a Jar, after this time, washed with deionized water, and for coloring the background for two minutes in a solution of haematoxylin were placed. After washing with running water, dried in the open air, target cells by light microscopy to identify specific and micrometer measurements were examined and photographed.

Periodic acid-Schiff staining

In this method, fixation solution by mixing 5ml of formaldehyde with 45ml of ethanol 95° C was produced, the slides were exposed for one minute in a solution to the fixing operation to be performed, the slides were washed in running water for one minute and then placed 5 minutes in Periodic acid solution, the slides were washed well with distilled water. 15 minutes were Schiff solution, next 5min washing with running water, and in painting background were 90 seconds in a solution of haematoxylin, slides were washed in running water must be drained, target cells by light microscopy to identify species and micrometer measurements were examined and photographed.

Alkaline phosphatase

- 1 - The first 45 ml of distilled water to bring the temperature 18 to 26 ° C.
- 2- Then we prepared diazonium salt solution in which 1ml of sodium nitrite are added to 1ml of alkaline FRV.
- 3- And then the salt solution prepared in distilled water which had been prepared in the first step are added.
- 4- Then 1ml Naphthol AS-BI to the diazonium prepared salt solution adding, and it into a glass jar poured, and well incorporate.
- 5- The sample is poured into jars and we wait 30 seconds and then the samples were washed with distilled water for 45 minutes, and then bring to the slides well dry, after all the samples in the baseline alkaline solution for 15 minutes, so stay from direct light, to get a good color, because these colors are sensitive to light and the light loses its enzymatic activity and the disabled, after 15min of incubation for 2 minutes were well washed with distilled water and bring to the dry slides, and then microscopic we evaluate the samples.





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RESULTS AND DISCUSSION

Diagnosis of leukemia, to predict disease status and choice of treatment in all cases with the morphological characteristics of the cells was not possible therefore, to reach the correct diagnosis Cytochemical tests and immune cell phenotype and cytogenetic studies are needed, knowing documentation Razi hospital in Rasht city in the context of sampling and in coordination with the previous was admitted to the hospital and from those for blood disorders (leukemia) were referred for biopsy, biopsy was performed. Patients were randomized to and the cluster approach 11 slides from each patient (a total of 102 slides), blood samples were taken immediately after sampling and drying the slides fixation solution, all the samples were fixed. Peripheral blood was taken from each sample, 11 slides were prepared all slides were coded by a diamond pen and then dried. Slides temporary with a fixation solution that is 1 volume of ethanol, 1 volume of methanol, and 3 volumes of acetone was prepared then fixed and then slide boxes filling and we've moved to the Laboratory of Immunology Lahijan Azad University Branch to investigate the pattern of leukocyte 1 gram of series slides with toluidine blue stained method, according to the morphology of the white blood cells, staining was white blood cells, in the population noted under study is preliminary. So if in collecting samples there is corruption so check white blood cells is inefficient. To examine patterns and differential diagnosis of adult cells based on pattern recognition Classic 5 cells, white blood cells, Cytochemical staining Naphthol AS-D chloroacetate esterase, Alpha-naphthalen propanoic acetate esterase, acid phosphatase, Periodic acid-Schiff staining Using the books of commercial construction company Sigma-Aldrich was used.

Following results were obtained of the 19 samples analyzed by Cytochemical staining, Alpha-naphthalen propanoic acetate esterase 15/75 percent positive, and 84/21 percent were negative. and 12 samples by acid phosphatase staining was found 25% of the samples over the color positive and 75% were negative, and also the 25 samples that were analyzed by alkaline phosphatase staining, 100% of the samples were negative, and 34 samples were examined by staining Periodic acid shifts 97/05% of the negative and only 2/94 percent of the samples were positive, and from 12 samples by Naphthol AS-D staining was evaluated 16/66% positive and 83/33% of the samples were negative, in general it can be concluded staining was performed for acute lymphoblastic leukemia. Single alpha-naphthalen propanoic acetate esterase, acid phosphatase, Periodic acid-shift, and Naphthol AS-D can show a positive reaction, that would be the staining are specifically used for the diagnosis of acute lymphoblastic leukemia.

Cytochemical staining general results obtained indicate that it was:

Alpha-naphthalen propanoic chloroacetate or specific esterase (AChE) which acute lymphoblastic leukemia is negative, but in some cases of chronic lymphoid leukemia is positive. PAS Stained, often with a rough granular cytoplasm has been PAS-positive acute lymphoblastic leukemia. The positive reaction of the acid phosphatase is localized in 20% of cases of ALL can be seen, that the origin of leukemic T cells (T cell ALL) implies.

ACKNOWLEDGMENTS

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Table1:Cytochemical characteristics in acute lymphoblastic leukemia		
Color Type	positive reaction	negative reaction
ANAE	15/78	84/21
APH	25	75
ALPH	0	100
PAS	2/94	97/55
NASD	16/66	83/33

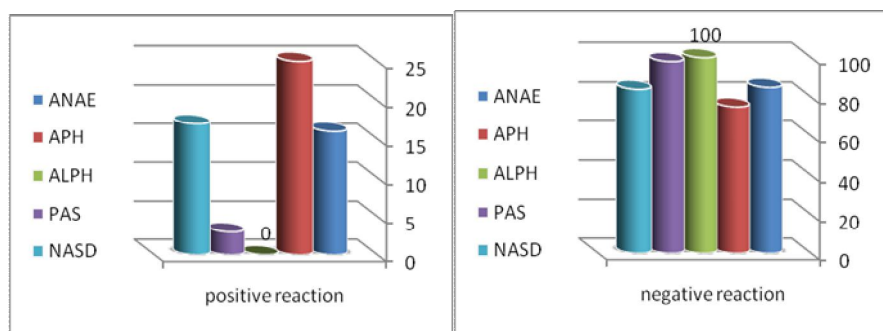


Figure 1: Cytochemical staining of positive and negative responses in lymphoid cells





Response of Maize (*Zea Mays L.*) to Foliar Application of Zinc Nutrient under Water Stress Condition

Amin Farnia^{1*} and Mohammad Amir Feyzi²

¹Assistant professor of department of agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.

²M.Sc student of department of agriculture, Boroujerd branch, Islamic Azad University, Boroujerd, Iran.

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*Address for correspondence

Amin Farnia

Assistant professor of department of agriculture,
Boroujerd branch, Islamic Azad University,
Boroujerd, Iran.

E-mail: Aminfarnia@yahoo.com



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ABSTRACT

To determine the effects of water stress and foliar application of Zinc nutrient on yield and yield components of maize a field experiment was conducted on a loam clay soil, in Islamic Azad University of Boroujerd, Iran, during the growing seasons 2013- 2014. The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were different irrigation periods (7, 14 and 21 days) in main plots and foliar application of Zn sulphate, Zn chelate, water and non application of them in sub plots. Foliar use of treatment was apply in two stages (6 leaf and before of flowering). The results of present study reviled that the effects of water stress and Zn nutrient treatments on all traits were significant but the interaction effect of them on 100grain weight and biomass and grain yield were significant only. Simple mean comparison of traits showed that 7 day irrigation period and foliar application of Zn sulphate treatments had the highest plant height, number of row per cob, number of grain per cob and harvest index and 21 day irrigation period and non-application of any Zn and water treatment had the lowest of them. However, highest 100-grain weight, grain yield and biomass yield were founded in foliar application of Zn nutrient in 7 day irrigation period and lowest of them were founded at non-application of any Zn and water treatment in 21 day irrigation period. The present study concluded that maximum production of maize was recorded for non stress treatment and was followed by application of foliar application of Zn nutrient, while severe water stress produced minimum



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production. Also, results of these experiment showed that application of Zn fertilizer had better effect on grain yield, yield components compared to the control. However, application of Zn sulphate that had highest production of maize is better than Zn chelate and water. Therefore, we can increase yield and yield components of maize by decrease of irrigation period and foliar application of Zn sulphate and Zn chelate spacialy Zn sulphate.

Key words: Irrigation, maize, water stress and Zn sulphate

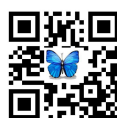
INTRODUCTION

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

Drought stress causes deceleration of cell enlargement and thus reduces stem length by inhibiting inter nodal elongation and also checks the tillering capacity of plants (Ashraf and O'Leary, 1996). Several studies have also shown that optimum yield can be obtained with irrigation at branching, flowering and pod formation stages (Prihar and Sandhu, 1968). Dry stress may cause some problems in absorbing process or the process of nutrient accumulation, this can not only lead to fertilizer loss but also causes decrease in yield of grain and plants (Chogan et al, 2004). Biglouei et al (2007) reported that the increase of drought stress in K.S.C.704 corn, led to increase of grain yield and protein percentage. They also declared that grain protein in irrigation treatments after 50, 75 and 100 percent water depletion, were relatively 5.8, 7.2 and 7.4. The studies of researchers have indicated that, lack of organic materials and existence of alkali reaction in calcareous soils can lead to lack of micronutrients in these soils (Auge, 2001).

Zinc is considered to be one of the smallest nutritious elements for many organisms. Nearly 200 enzymes and transcription elements of zinc need it as one of the most essential components. Zinc plays an important role in protein and carbohydrates syntheses. It also has effects on growth of stem and root (Kabata-Pendias, 1999). Zinc plays an important role as a metal component of enzymes (alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural, or regulator cofactor of a large number of enzymes (Marschner, 1986). Mahady (1990) found that foliar application of Zn SO for faba bean plants increased number of pods/plant and seed yield/fed. Guenis et al. (2003) and Soleimani (2006) reported marked increase in number of grains spike-1 of wheat for foliar application of boron and zinc, respectively. Soleimani (2006) reported increase in biological yield for foliar application of zinc. Torun et al. (2001) and Grewal et al. (1997) reported increased wheat production with application of zinc and boron over control. Grain protein content and baking quality highly depend on genetic background and environmental factors, especially influence of drought and heat stress, during the grain filling period (Luo et al., 2000; Ottman et al., 2000). Marshner (1993) reported that, by increasing consumption of Iron and zinc in corn, we can witness that the total amount of carbohydrate and grain protein is increased, and as a result the gain weight, number of grains and at last yield will be increased as well. Tahmasebi et al (2003) declared that by increasing amounts of zinc, absorption of nitrogen will be decreased by the plant, yet potassium absorption will be increased. They also concluded that the most balanced status between nitrogen, phosphor and potassium absorption with 20 kilograms zinc sulfate per hectare was observed in saline soils. Fecencko and Lozek (1998) studied the effect of zinc sources on yield, protein percentage and macronutrient absorption in corn, and they witnessed that by three years of manure, the average amount of grain protein was significantly increased.

Therefore this study was planned to examine effect of drought stress and Zinc fertilizer on yield and yield components of maize.



**Amin Farnia and Mohammad Amir Feyzi****MATERIALS AND METHODS****Field material and Experimental design**

This study was carried out in order to evaluate the effects of drought stress and Zinc fertilizer on yield and yield components of maize in the faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch (experiment station: Hamedan), Iran during the growing seasons 2013- 2014. Soil property of experimental field showed in table1.

Treatments

The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were different irrigation periods (7, 14 and 21 days) in main plots and foliar application of Zn sulphate, Zn chelate, water and non application of them in sub plots. Foliar use of treatment was apply in two stages (6 leaf and before of flowering).

Yield and yield components determination

In this field experiment there were 6 rows in each plots and rows were 6 m long with 0.75 m row spacing and plant to plant spacing was 18 cm too. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle 3m² of the four 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 3m² of the four central rows in each plot. 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 harvest index was accounted with follow:

$$HI = (\text{Economical yield} / \text{Biological yield})$$

Statistical analysis

The statistical analyses to determine the individual and interactive effects of treatments were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

RESULTS AND DISCUSSION

Plant height: The analysis of variance showed that, the effect of water stress and Zn nutrient on plant height were significant only (table 2). The comparison of the mean values of the plant height showed that 7 day irrigation period treatment had the highest (210cm) plant height and 21 day irrigation period treatment had the lowest (160cm) plant height. In foliar application of Zn treatments Zn sulphate treatment had the highest (195cm) plant height and control treatment had the lowest (176cm) plant height and the differences were significant (table 3).

Number of row per cob: The effect of water stress and Zn nutrient on number of row per cob was significant (table 2). The comparison of the mean values of the number of row per cob for water stress showed that 7 and 14 day irrigation period treatments had the highest (16) number of row per cob and 21 day irrigation period treatment had



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the lowest (13) number of row per cob. In foliar application of Zn treatments Zn sulphate treatment had the highest (16) number of row per cob and control treatment had the lowest (13) number of row per cob and the differences were significant (table 3).

Number of grain per cob: The effect of water stress and Zn nutrient on number of grain per cob was significant (table 2). The comparison of the mean values of the number of grain per cob for water stress showed that 7 day irrigation period treatment had the highest (500) number of grain per cob and 21 day irrigation period treatment had the lowest (380) number of grain per cob. In foliar application of Zn treatments Zn sulphate treatment had the highest (470) number of grain per cob and control treatment had the lowest (395) number of grain per cob (table 3).

100 grain weight: The results showed that the effect of water stress, Zn nutrient and interaction between them on 100-grain weight were significant at 1% level (table 2). The comparison of the mean values of the 100-grain weight for interaction between water stress and Zn nutrient foliar application Zn sulphate in day irrigation period treatment had the highest (26g) 100-grain weight and non application of Zn in 21 day irrigation period treatment had the lowest (14g) 100-grain weight (figure 1).

Biomass yield : The effect of water stress, Zn nutrient and interaction between them on biomass yield were significant at 1% level (table 2). The comparison of the mean values of the biomass yield for interaction between water stress and Zn nutrient foliar application Zn sulphate in day irrigation period treatment had the highest (19000kg.ha⁻¹) biomass yield and non application of Zn in 21 day irrigation period treatment had the lowest (9000kg.ha⁻¹) biomass yield (figure 2).

Grain yield: The results showed that the effect of water stress, Zn nutrient and interaction between them on grain yield were significant at 1% level (table 2). The comparison of the mean values of the grain yield for interaction between water stress and Zn nutrient foliar application Zn sulphate in day irrigation period treatment had the highest (10750kg.ha⁻¹) grain yield and non application of Zn in 21 day irrigation period treatment had the lowest (2350kg.ha⁻¹) grain yield (figure 3).

Harvest index (HI): The effect of water stress and Zn nutrient on harvest index was significant (table 2). The comparison of the mean values of the harvest index for water stress showed that 7 day irrigation period treatment had the highest (47%) harvest index and 21 day irrigation period treatment had the lowest (28%) harvest index. In foliar application of Zn treatments Zn sulphate treatment had the highest (44%) harvest index and control treatment had the lowest (31%) harvest index (table 3).

The results of present study revealed that the effect of water stress and foliar application of Zn on all traits were significant but the interaction effect of them on 100grain weight and biomass and grain yield were significant only (table 2). Water stress is deleterious for plant growth, yield and mineral nutrition (Garg et al., 2004; Samarah et al., 2004). Soil moisture status during the reproductive phase of crops plays an important role to determine the impact of yield component in final grain yield (Singh and Bhushan, 1980). This study has showed that maize grain yield and yield components decreased significantly with the increase of irrigation period. The reduction in plant height, number of row per cob and number of grain per cob under water stress treatments may be attributed to the limitation of dry matter partitioning to the reproductive sink or even grain formation factors as has been reported by Turk et al (1980). The significant reduction in number of total grains in plant under drought stress may be attributed to the abscission of the reproductive structures. Ziska and Hall (1983) and Gwathmey and Hall (1992) reported similar results. The number of grain per plant in 7 day irrigation period giving a 22% increase over the 21 day irrigation period.

The grain yield in the water stress condition was restricted by limited moisture availability. Drought occurrence in relation to anthesis stage causes a drastic reduction in yield and yield components (Seghatoleslami et al., 2008).



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However, the results showed that under 7 day irrigation period conditions significantly gave better grain yields than 21 day irrigation period conditions. The grain yield of maize in the 7 day irrigation period and application of Zn sulphate treatment had a 10750kg.ha⁻¹ that is 5 equivalent of grain yield in non application of any foliar nutrient and 21 day irrigation period (figure 3). Pandey et al (2002) stated that the maximum water consumption by the corn is almost when silk rating or immediately after that. Water deficit when tasselling and silk rating along with decreased grain number per ear decreases grain yield the most. Therefore, the main cause of decreased grain yield in drought stress treatments seemed to be significant decrease in grain number per cob. Thaloath et al (2006) reported that zinc spraying under water stress conditions, had positive effect on growth, yield, and yield components of plants. The findings of this experiment conformed to the results obtained by Sheykhbagloo et al (2009). This result was also supported by the other researchers (Mojadam, 2006 and Lack, 2006).

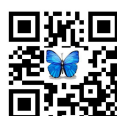
Folier application of Zn nutrient had a positive effect on the grain yield and biomass yield and yield components of maize. In maize, the final grain yield is dependent on the number of cob per plant, number of grains per cob and the extent to which grains are filled. In the present study, the reduction in grain yield under water stress was associated with dramatic decrease in all yield components (Table 3). Supporting evidences were reported by many researchers (Ziska and Hall, 1983; Ludlow and Mushow, 1990; Gwathmey et al., 1992). They attributed the reduction in grain yield under water stress to the reduction in number of grain per plant, biomass yield and grain weight. Turk and Hall (1980) attributed the reduction in grain yield under drought stress to the secondary detrimental effects of drought avoidance on CO₂ assimilation. This result suggests that maize exhibit reproductive plasticity under drought stress conditions.

Decrease biomass yield under lower soil moisture might be due to reduction of leaf area and photosynthesis rate (Sinaki et al., 2007). Different irrigation period treatments indicate with increasing water stress the biomass yield decreased significantly. The biomass yield in the 7 day irrigation period and application of Zn sulphate treatment had a 50% over the non application of any foliar nutrient and 21 day irrigation period treatment (figure 2). Latiri-Soki et al (1998) reported that, irrigation and fertilizers increased biomass yield and grain yield. They suggested the increase might be due to increased leaf area index (LAI) and an increase in the period for which the crop remained green which resulted in increased capture efficiency of radiation energy and consequently more dry matter production.

In the present study harvest index was dramatically decreased with increased in irrigation period and non application of any Zn nutrient treatments. Also, Ziska and Hall (1983) founded that the effect of water stress on HI to the reduction in assimilate supply attributed. This suggests that maize which application of Zn nutrient gave higher grain yield under water-stressed conditions could play an important role in sustaining crop production in semi arid regions.

CONCLUSION

The present study concluded that maximum production of maize (grain yield, yield components and grain yield) was recorded for non stress treatment and was followed by application of foliar application of Zn nutrient, while severe water stress produced minimum production. Also, results of these experiment showed that application of Zn fertilizer had better effect on grain yield, yield components compared to the control. However, application of Zn sulphate that had highest production of maize (grain yield, 100 grain weight and grain protein yield) is better than Zn chelate and water. Therefore, we can increase yield and yield components of maize by decrease of irrigation period and foliar application of Zn sulphate and Zn chelate spacialy Zn sulphate.





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Table 1. Soil property of experiment site.

soil Texture	sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (ppm)	N (%)	pH	EC (ds/m)	Depth
LC	20	45	35	220	8.2	0.1	7.7	0.409	0-30





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Table2. Analysis of variance (mean squares) for yield, yield components of maize under water stress and Zn nutrient

Treatments	df	Plant height	number of row per cob	number of grain per cob	100 grain weight	biomass yield	grain yield	harvest index
R	2	1229	1.39	2472	0.18	8608201	737705	108
Irrigation (A)	2	8138**	33**	78257**	381**	213078163**	136727622**	2403**
Ea	4	209	0.35	3945	10.6	2556246	1656643	33
Zn (B)	3	371**	17.1**	12541**	38**	40293589**	23273883**	274**
A*B	6	68	0.82	960	1.7**	2275190*	1426592**	43
Eb	18	56	0.83	1319	0.49	802402	342387	27
CV(%)		4	5.9	8.3	3.88	6.4	10.3	13

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

Table 3. Simple mean comparison for yield, yield components of maize under water stress and Zn nutrient

treatment period	Plant height (cm)	number of row per cob	number of grain per cob	harvest index (%)
Irrigation period				
7 day	210a	16a	500a	47a
14 day	190b	16a	440b	41b
21 day	160c	13b	380c	28c
Zn				
Zn sulphate	195a	16a	470a	44a
Zn chelate	190ab	15a	460a	41a
water	184bc	14b	420b	33b
non application	176c	13b	395b	31b

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





Amin Farnia and Mohammad Amir Feyzi

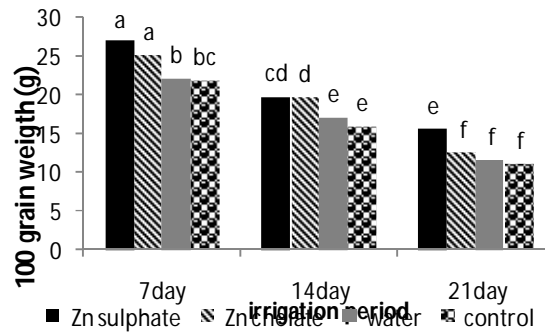


Figure 1. Effect water stress and Zn nutrient on 100 grain weight in maize.
Means by the uncommon letter in each column are significantly different (p<0.05)

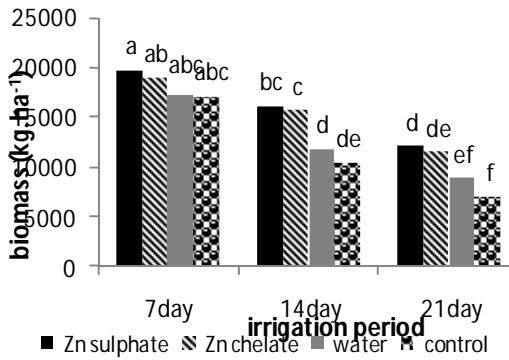


Figure 2. Effect water stress and Zn nutrient on biomass yield in maize.
Means by the uncommon letter in each column are significantly different (p<0.05)

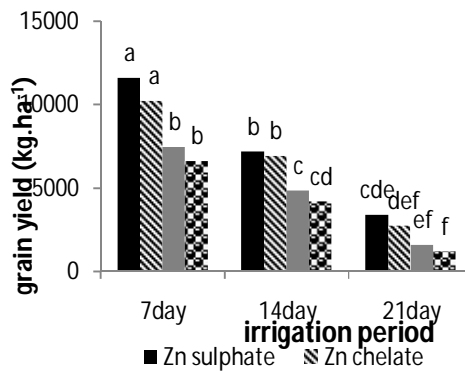
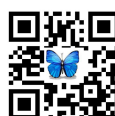


Figure 3. Effect water stress and Zn nutrient on grain yield in maize.
Means by the uncommon letter in each column are significantly different (p<0.05)





RESEARCH ARTICLE

The Comparative Study of the Condition of Trading of Bankrupt Merchant in Iran and Egypt Law

Halimeh Ghaffari*, Seyed Hossein Sadat Hosseini and Mohammad Javad Baghizadeh

Faculty of Private Law, Damghan Branch, Islamic Azad University, Damghan, Iran.

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*Address for correspondence

Halimeh Ghaffari
Faculty of Private Law,
Damghan Branch,
Islamic Azad University,
Damghan, Iran.



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ABSTRACT

Namely the trading of bankrupt merchant is done in three periods: Trading before suspension date: The trading of this period is based on validity. The trading after stop and before issuing the order: The law maker in article 423 in three sections define the trading nullified. The trading after the order issue: Despite some of the law makers that know the trading of this period as nullified, the trading of this period is correct (enforced or not enforced) or it is nullified. The present study investigated the condition of bankruptmerchants in three sections with investigation of the rules of this period and adjusting them with Egypt law. The present study to achieve the study goals applied descriptive-inference method.

Key words: Bankruptcy, Debt, Merchant, prohibited interference, suspension.

INTRODUCTION

All people in their life are faced with economic problems and this causes the lack of affordability of paying the debts. This problem is more possible among merchants and based on the credit principle of merchant trading, it affects many people. In Islamic jurisprudence, in case of the lack of affordability in paying the debts, they will be included in destitution law. In Islam law, there is no difference between merchant and non-merchant, if a person debt is more than his asset, he is destitute and in case of its proof to the ruler and issuing destitution order, he cannot intervene in his own property.

By approving law principles approved in 1329, destitution meaning in sharia is considered by the law maker. In 1310, destitutionand insolvency law were approved in which insolvency was recognized as a new phenomenon beside



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destitution. Destitution and insolvency law was abolished based on insolvency law approved in 1310 and since then legal establishment of destitution and insolvency law was removed totally from the rules. Based on article 39 of insolvency law: "from the enforcement of this law, no claim named destitution claim is not accepted". Later, the law makers applied the destitution and destitute terms in some rules as articles 1264, 1265 of civil law approved in 1314 and it is mistaken and it should be modified (Jafari Langerudi, 1996).

For the first time, the rules of bankruptcy were approved in 1303, 1304 inspired by France. In this law, there is a difference between merchant and non-merchant. Then, trade law was approved in 600 articles in 1311. The law of clearance of bankruptcy was approved in 60 articles in 1318. Egypt being inspired by France and Belgium, advanced their commercial rules and in article 195, self-bankruptcy commerce law was considered for the merchant. The main problem of the study is the condition of bankrupt merchant trading in Iran law and Egypt?

Bankruptcy is based on the condition that merchant can not afford his debts but it is possible that the merchant has immovable assets but the basis of the law maker is paying his debts. There should be two conditions for declaring a bankrupt merchant: First, being merchant, second not paying the debts. The trading of bankrupt merchant is occurred in three stages, before suspension date or unclear period or in the period after issuing the order. The present study was investigated for better study and achieving the main problem solution of the condition of bankrupt merchant trading in three sections and the rules of these periods and its comparison with Egypt law were investigated. The main issues in Iran law are dedicated to articles 418, 424, 425, 426 and 557 and others are associated with the main cases. In Egypt trading law, the main cases of the study are in articles 589, 598, 599 and 238 and articles 2,3,4,5 in relation to the main items are also investigated.

Study methodology

The study method was descriptive-analytical and the aim of the researcher was describing and analyzing the data of documents, books and resources of hypotheses and study questions. The data were collected from library resources and documents. The data collection instrument in this study was taking notes from the books, journals and writing of legal sites.

The data of the present study was qualitative and based on the nature of data, they are classified, analyzed and inferred logically. Thus, the analysis of collected data was a response to the study problem by reasoning and inference method.

First section: Transaction before suspension date in Iran and Egypt law

The bankruptcy of the merchant or commercial company is occurred as the result of not paying the debts. In some countries, bankruptcy is only dedicated to merchants like Iran, an exception is cooperative company that although they are not merchants, they are included in bankruptcy rules (section 6, article 54 of cooperative companies). In Anglo-Saxon countries, both merchants and non-merchants are considered and in other countries, besides merchants, legal entities of private law are considered. Like Iran, in Egypt law in article 195 of commerce code, bankruptcy is specially for merchants. In trading before suspension date, the principle is based on validity. The exception is in two articles 424, 426 commerce code. In article 424 commerce code, it is said that If, as a result of a lawsuit by the liquidator or a creditor against those persons who have been a party to a transaction with the trader, or against their legal successors, it is proved that the trader who has ceased payment of its debts has made transactions to avoid his liability or to inflict loss on the creditors, such transactions can be annulled unless the other party to the contract pays the price difference before an annulment order is issued."





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The cancellation claim is accepted within two years from the date of the transaction in the court. Another exception is article 426 commerce code as: " If it is established in the court that the transaction is a sham or due to collusion, the transaction will be null and void, and the goods, as well as the gains thereof, must be returned. If the other party to the contract becomes a creditor, he will be treated as an ordinary creditor.

Revocable transactions

In article 424 commerce code, it is stated that "If, as a result of a lawsuit by the liquidator or a creditor against those persons who have been a party to a transaction with the trader, or against their legal successors, it is proved that the trader who has ceased payment of its debts has made transactions to avoid his liability or to inflict loss on the creditors, such transactions can be annulled unless the other party to the contract pays the price difference before an annulment order is issued."

For annulment claim for the date before suspension, four conditions are considered:

The transaction is before insolvency date.

Transactions with the intention of avoiding debt or creditors

Loss is more than one fourth.

From the transaction, two years is not passed yet.

Nullified transactions

If it is established in the court that the transaction is a sham or due to collusion, the transaction will be null and void, and the goods, as well as the gains thereof, must be returned. If the other party to the contract becomes a creditor, he or she will be treated as an ordinary creditor. Nullified transactions are sham collusion.

The investigation of article 426 of commerce code and article 218 civil code.

In accordance to article modified 218 article, order of nullification of transaction is issued when first, the transaction is done to avoid debt and second it is sham. Thus, both conditions should be proved to use the order. Thus, if the transaction is to avoid debt and it is not sham, this trade is valid and if the transaction is sham and it is not done to avoid debt, it is valid. These are two shortcomings of the revised article (GharibeKhunin, 1992, 74).

In commercial code of Egypt, there is not article about the transaction before bankruptcy order and based on section 1, see article 2 commerce code of Egypt.

At first regarding the agreement between two parties, otherwise based on commercial code or other articles in commercial law, or the customarily law of commercial code and then public law of this country, civil law can be considered. In Egypt law, see in article 238 of civil law.

If the debtor property is not gratuitous, the principle is based on its validity and if the debtor possession in for creditor is not enforced, the creditor should prove that this possession is based on sham or the debtor was aware of his insolvency (Ahmad Sahnevari, 2013).

The comparison of different types of transactions before suspension date

Transaction to avoid debt with sham transactions and there is public and private relations between them.

It is possible that transaction is sham but there is no avoidance of debt. Article 463 of civil law is an obvious example.



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It is possible that transaction is to avoid debt but it is not sham. Article 65 of civil law is an example in this regard. It is possible that transaction is both sham and it is for avoiding debt. Article 218 of civil law is an example.

Now, based on article 218 of civil law, section 3, transaction is nullified and section one order due to the lack of intention is void, it is considered in article 426 of commerce code as intention is one of the main principles of contract. In civil law, from article 218, we can not infer second section order but based on article 65 of civil law considered validity of the loss of creditor dependent upon the permission of creditor and based on it the order is issued. Article 65 of the contract has not feature that article 35 is specific for it. It can be said that the order of the article as article 218 is one of the items of performing this rule and transaction is not enforced.

Article 424 of commerce law emphasized on validity and referred to cancellation term in the mentioned article. If the transaction is to avoid debt before the suspension date, the transaction is cancelled in case of not paying the difference of price and it is enforced. According to Dr. Katuzian, cancellation in article 424 of commerce code is that transaction is not enforced from the conclusion date and it is not as benefits of property between the transaction and order of court is dedicated to court.

Second section: Transactions in suspension period

The bankrupt merchant not paying his debts is in a condition that all his legal acts are suspended and the law maker considers some of the transactions of the merchant as nullified or void.

Regarding the legal basis of nullification of these transactions, some of the authors considered banning transaction for the bankrupt merchant is the basis of nullification (financial, 1357, 35). However, other writers including Dr. Eskini (2005) considered the nullification of transaction based on not paying the debts and it is established to avoid sham of the debtor to eliminate the equal conditions between the existing creditors. We believe Dr. Eskini is right as the law maker didn't nullify all the legal acts of the bankrupt merchant and they can be nullified if there is sham to the creditors. In suspension period, the followings should be considered:

First part: Setting suspension date

Nullification of merchant transactions is determined from the date of declaration of bankruptcy and it is possible that the merchant perform transactions to inflict loss the creditors and suspension date of paying the debts is the criterion of considering nullified or valid transactions. This date is temporary. It is because exact determination of suspension date is difficult and it can be objected and it is limited to determine the validity of claims of creditors and after that no claim from creditors is accepted. But liquidator can ask the court to change the suspension date, if necessary. Changing suspension date in commerce code of Egypt from the court or from the creditors or debtor or beneficiaries is determined whiting 10 days of approval of the claim of creditors from the court (563 commerce code of Egypt).

Second part: Declaration of suspension date

The merchant should declare suspension from the date of suspension. Based on article 413 commerce code, "The merchant should declare its bankruptcy within three days of the date of the suspension in the payment of its debts or other obligations to the court office of the place of its residence". According to most of the law makers, the three days is very short time for merchants namely great commercial companies to be suspended. This period in commerce code of Egypt (553 commerce code) is 15 days after the suspension date. Although this period is also short for great commercial companies from the view of Egyptian law makers to determine suspension (Albarudi, 1977, 278), it is more than the law of Iran.



**Halimeh Ghaffari et al.****Third part: Suspension date**

The distance between suspension and issuing bankruptcy order in Iran is not definite and the judge should define suspension date in his order and if the judge doesn't determine suspension date in his order, the date of issuing order will be the suspension date. Thus, the court can determine suspension date of the bankrupt person even years before this period. Therefore, most of the transactions are problematic. But in France law, as our law is inspired by it, this period is 6 months and this is necessary for the stability of transactions (SotudeTehrani, 1971).

In Egypt commerce code, see the first section article 562.

But if the court doesn't set the suspension date in its order, the order issuance date is the suspension date. The problem of Iran law is the consistency of suspension and order issuance date and the different effects of these two periods. In Egypt commerce code it is true and in section 3, article 563. In commerce code of France, the maximum period before bankruptcy order issue considered as suspension date from paying the debts is six months before the order issue and this limited period is necessary for stability of transactions and foreign traders can easily trade with such country as finding about the suspension state of the merchant is difficult. This period in commerce code of Egypt is 2 years and although this period is more than that of France code, according to Egyptian law makers, the transactions are not stable (Naaeim, 1996, 273). But it is better compared to Iran code that no limitation is considered in this regard.

Third section: Transactions after order issue

Immediately after issuing bankruptcy order, without it is necessary to notify the order to the bankrupt, will of the merchant is suspended in managing his property and the order has public effect and it is not about the specific property of the merchant.

Law maker in article 418 commerce code is said:

"The bankrupt merchant, from the date of the bankruptcy order, is prohibited from any interference in its property, including financial gains accrued during the bankruptcy proceedings.

Also, it is said in the article that:

The liquidator is the legal substitute for the bankrupt trader with all the powers and financial rights, the exercise of which is necessary to facilitate the payment of its debts, and has the right to use the said rights on his behalf".

All transactions of the merchant after bankruptcy order is null and void and it is not restricted to article 423 of commerce code as article 418 commerce code stated merchant prohibited interference in his properties and the result is nullification of the transactions of the merchant after bankruptcy order.

The reasons of followers of nullification of transactions of the bankrupt merchant

For the nullification of the transaction of the bankrupt merchant, there are two reasons. The first reason in accordance with article 423 of commerce code and the problem of this reason is that it didn't consider the scope of article 423. It means that in this article, only some of the transactions of the merchant are nullified before issuing order and after suspension. For example, when the merchant does Gratuitous transfer of property or pay the debt to the creditor, from an order that is limited to some special items, a general order is extracted. As such comparison is not extracted from article 423 and its inclusion to all the transactions after order issue is not consistent with the related principles.



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But regarding another group who say respecting the order of law maker indicates that transactions of the bankrupt merchant is nullified after the order issuance. According to the regulations of jurisprudence, banning the worship leads to corruption but banning transactions doesn't lead to corruption. For example, jurists say don't say prayer with dirty clothes. When a person says prayers with dirty clothes, his prayer as worship is false. Banning the transactions doesn't lead to nullification. For example, the jurist say don't trade in Masjeloharam. Does it mean that if you traded, your transaction is nullified or it means that you should respect this place and it is not a place for trade.

Most of the jurists believe that banning the transactions doesn't lead to nullification. Thus, if we consider this issue in commerce, if the law maker in article 418 of commerce code, prohibited the transactions of the bankrupt merchant after bankruptcy order, the meaning of prohibition is not the nullification of the transaction. In addition, it can be said that not nullifying the transactions of bankrupt merchant after order issue and non-enforcement of it helps restoration of public order but its nullification is not so.

There are some sections in this regard that are explained later.

First part: Destitutionoption in bankruptcy

The cancellation of transaction with the bankrupt merchant is considered in the section of return claim in articles 528-535 of commerce code. Returning property or documents from bankrupt merchant has no unit basis. In articles 528,529, the owner of documents or goods, ask for returning the documents or property of the merchant. In articles 530-534 of commerce code, returning the property is considered that is transacted before. Article 530 of commerce code is stated: " The goods being purchased by bankrupt merchant for another account, if the price is not paid, it can be returned from the seller otherwise from the person the goods are purchase from his account".

The merchant here is a **commission agent** who accepts or sells goods for the account of another person, second as he is the main buyer, the transaction is registered by him and in accordance with article 357 of commerce code, "**commission agent** accepts or sells goods for the account of another person, but in his own name and he received his commission".

Second part: Prohibition of interference

Bankrupt merchant is prohibited from interference in his property after order issue and article 418 of commerce code is absolute and it includes all his property.

According to article 418, commerce code, if after bankruptcy order, the merchant inherits money that his assets are increased, if the bankrupt merchant accepts the money implicitly or explicitly, this increase of money is divided among the creditors. But if the merchant didn't accept the money as in our legal system, legacy is compulsory, and to reject the legacy, a period of one month is considered and it is started from the awareness of heir of the death of the dead person and if he doesn't reject the legacy, it is stated as accepted and it is given to the creditors from the liquidator and the merchant is prohibited to interfere.

Third part: Warranty of performance of interference prohibition

If after bankruptcy order issue, a transaction is performed as in article 423 commerce code, as the transactions before order issue are nullified they are nullified after order issue.



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Law department of justice office on 52/10/16 in consulting view declared all the transactions after order issue nullified and stated that "What is mentioned in article 423 commerce code about nullification of some of the transactions of bankrupt merchant is dedicated to after suspension and before order issue otherwise, the bankrupt merchant, from the date of the bankruptcy order, is prohibited from any interference in its property, including financial gains accrued during the bankruptcy proceedings. liquidator is the legal substitute for the bankrupt trader with all the powers and financial rights, the exercise of which is necessary to facilitate the payment of its debts, and has the right to use the said rights on his behalf". Any transaction of the bankrupt merchant after bankruptcy order is null and void.

Fourth part: Conclusion of composition contract (article 491 commerce code)

If the bankruptcy of the merchant is finished by each of the mentioned items, the limitations of bankruptcy order, personal claim of prohibition of interference of the merchant in his property is eliminated but the term bankruptcy and social deprivation dedicated to the bankrupt merchant are eliminated only by his past fame and credit.

Most of the law makers believe that all the transactions of the merchant after bankruptcy order are nullified. The exception here is article 500 commerce code as: In the transactions the bankrupt merchant approves the composition agreement till the nullification or cancellation order issue, it is not nullified unless it is stated that it is for infliction of loss to the creditors.

The opposite concept of this article is the merchant who obtained composition contract after the bankruptcy order, his transactions are valid, unless it is stated that he had not good intention and it is nullified and second if he doesn't conclude composition agreement, his transactions are null and void.

CONCLUSION

Article 589 of Egypt law said: After destitution order is issued, the property of destitute is taken out of his possession and he cannot interfere in his property from the order issue date.

Unlike Iran, in this article, it is stated about destitution and it shows that Egypt law unlike Iran law considered the lack of affordability. This issue in Iran commerce code is as by not paying only one debt, you are declared as bankrupt even his assets are enough to pay the debts and this is not good for the fame of the merchant.

Article 424 commerce code

Article 424 commerce code predicted the transactions to avoid debt or inflicting loss to the creditors. This article has special conditions as the transaction can be nullified.

The conditions are including:

The transaction is before insolvency date.

Transactions with the intention of avoiding debt or creditors

loss is more than one fourth.

From the transaction, two years is not passed yet.

Regarding article 424, commerce code, it is assumed that the person in the transaction had good intention and he entered the trade only to acquire more profit but the merchant had bad intention. Thus, if the loss is one fourth or less, as in customarily it is ignored, the transaction is not nullified or if the price difference is paid before the order



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issue, the transaction is not nullified and if the price is not paid, he will not be treated as an ordinary creditor and liquidator should pay the price before dividing the property of the merchant among the creditors. In gratuitous transaction, the law maker doesn't accept one fourth of loss and indeed he doesn't accept in gratuitous transaction that loss is more than one fourth. The transaction done to escape the debt or inflict loss to the creditor has no problem in the main principles and its motivation leads to the invalidity of the transaction. In article 424 commerce code, the nullification term is not consistent with the lack of enforcement. That term in article 424 commerce code is more close to the lack of enforcement in civil law principles.

Article 426 commerce code

In collusion transactions, the two parties conclude the contract against the law and unlike the sham transactions, in this type of transaction, the two parties have will and second they concluded the required transaction, transaction in which the type and object of transaction and the parties are definite. Thus, we can not know these transactions equal to the sham transactions that due to the lack of intention or intention contraction are nullified. On the other hand, the law maker is blamed that they had no distinction regarding the transactions before the suspension date between the state in which the parties of the contract are aware of the bad intention of the merchant or not and they stated that both cases are some examples of article 424 commerce code and under difficult terms, the transaction nullification is ordered.

If the bankrupt merchant despite his awareness of financial crisis, do some transactions to inflict loss the creditors and the contract party is aware of his condition and his bankruptcy, this is collusion and is included in article 426 commerce code and the contract party has bad intention and not only he can not pay the difference of price and avoid the transaction nullification and receive the money, he is faced with nullified transaction and he should be included in the creditors group and in case of the lack of awareness, he is included in article 424 commerce code.

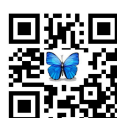
The distance between suspension and bankruptcy order in Iran is not definite and the judge should specify the suspension date, otherwise the order issue date is the suspension date. Thus, the court can specify suspension date of the merchant even some years before this period. Thus, most of the transactions are instable. But in France law, this period is considered as 6 months and this is necessary for the stability of transactions.

The current commerce code in article 418, 423, commerce code considers bankrupt transactions nullified and such transaction based on the nullification rules is not nullified as the real meaning of nullification technically, is the lack of occurrence of transaction. When it is said that the transaction is nullified, it means that the transaction is not occurred and there is no legal effect, the transaction of insane, nullified transactions in future from the contract parties and their legal agent is not approved.

It can be said that the philosophy of invalid transactions is not due to the inherent insanity or lack of intention or illegality for transaction, it is due to keeping the rights of creditors. Thus, the creditors can enforce the transactions. Thus, when it is said that the bankrupt merchant transaction is not enforced, it is true and as France law considered relative nullification in these cases that is close to the lack of enforcement.

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RESEARCH ARTICLE

The Effect of Institutional Factors on Foreign Direct Investment in Developing Countries

Mohammad Dalmanpour¹ and Akbar Komijani^{2*}

¹PhD student of Economics, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Professor of Economics, Faculty of Economy, University of Tehran, Tehran, Iran.

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*Address for correspondence

Akbar Komijani
Professor of Economics,
Faculty of Economy, University of Tehran,
Tehran, Iran.
Mobile: +98 9122384055
E-mail: komijani@ut.ac.ir



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ABSTRACT

The purpose of this study is to examine the effect of institutional factors of economic freedom, economic security and the voice and accountability beside the economic factors on foreign direct investment by GMM (Generalized Method of Moments) of dynamic panel data in 114 developing countries for the period 2004-2011. This method is the most efficient ways to estimate the effectiveness of institutions. Because this method solves the problems of endogenous institutional index. In this study, three groups of developing countries, countries with a high human development (40 countries), countries with a medium level of human development (35 countries), countries with a low human development (39 countries) have been classified.

Results show that the Institutional factors of economic freedom, economic security and the voice and accountability have significant positive effect on attracting foreign direct investment in developing countries with a high human development .However, in the other two groups of countries (with weaker institutions), institutional factors have adverse effects on attracting FDI. This shows that all countries, especially developing countries should make institutional reform to attract more foreign investment in various sectors.

Key words: Institutions, foreign direct investment, panel data, GMM



**Mohammad Dalmanpour and Akbar Komijani****INTRODUCTION**

Ongoing process of development and growth in the economy needs the precision in its creative factors. According to economic theory, investment is one of the effective factors on economic growth. Also foreign direct investment is one of the major substrates for increasing economic interaction in the international arena. In this regard, the provision of the required economic capital is always one of major concerns. Because of limited domestic capital, foreign capitals are used as a supplement to finance economic plans. Foreign direct investment is one of the types of foreign investment which all countries compete to attract it more and more in recent years.

FDI inflow in addition to financing, led to technology transfer, expertise human capital, knowledge and management. So in order to achieve sustained economic growth and stability it is necessary that identify the effective factors on FDI scientifically.

Although the foreign direct investment has grown considerably since the 1980s in the global level, but most part of the process of developing countries were not significant. Economists considered many factors as the main reasons for the performance differences in attracting the FDI between different countries to response the inability of some countries to attract FDI. Some factors show a part of observed differences across countries in attracting FDI such as, the size of economy, exchange rate, inflation rate, the intensity of trade openness, wage rates, the ratio of foreign debt to GDP, the ratio of domestic investment to GDP and etc. But certainly there are numerous factors have been neglected in the minds of economists so far. The role of institutions in attracting the FDI is one of factors which have far less attention. In this paper, institutional factors are: economic freedom, the voice and accountability, and economic security which have been studied beside the other economic effective factors on foreign direct investment in developing countries. The sample of developing countries is for 2011 to 2004.

LITERATURE REVIEW

There are strong reasons to attract more foreign direct investment in the environments or countries with strong institutions (including effective bureaucracy, low corruption, regulation, proper implementation of contracts, etc.). This chapter discusses the role of institutional factors in determining the FDI and weaknesses and deficiencies in the empirical research. Thus, a proper understanding of the relationship between institutions and foreign direct investment is presented.

Institutionalisms pointed out the weaknesses of neoclassical and ignore the value of economy and issues related to low levels of salaries and income distribution and raise the civil institutions creation to solve the problems of classical and neoclassical schools. The institutional economics, believes that the economy should be the optimal combination of market institutions, government and civil institutions. The optimum composition should be such that minimizes the exchange cost of private contracts (among economic agents) and free ride (at collective decisions) . Institutional studies indicate that many institutions and institutional factors do not exist in developing countries and if they are available they're not effective. In the absence of appropriate institutions, motivations have no impact. Therefore markets need non-market institutions support.

Institutional perspective believes that if some or all the neoclassical assumptions are not true, our analysis is wrong. If the institutions do not exist in neoclassical theory, predictions will be wrong. Neoclassical economics is based on the institutional framework assumption and its strong point is that if there is an institutional framework, it is maintained at all times and places. However, the weakness point of this factor is that if there isn't any institutional framework, not only it cannot tell us that no one can predict the cause of the failure but also it hasn't enough ability





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to track the problem. We should know appropriate institutions in neoclassical theory to have a better analysis. Therefore institutions play an important role in the theoretical analysis and better understanding.

Thorsten Veblen defined the economics as the processes and practices detection, and known that institutions shape the process. He believed that institutions can effect on the behavior of economic agents and economic performance. This influence is done by multiple channels which emphasizes on the role of institutions. Institutions have dominant effect on the structure of economic incentives in society. Economists believe that institutions influence on the limit of people's selections in society.

Theoretical explanation of the relationship between foreign direct investment and institutional quality can answer fundamental questions of economic planners, especially in developing countries. Because these countries were suffering from low level of investment and consequently lower per capita income and the income gap extension.

Institutions and foreign direct investment: a theoretical approach

To better understand the role of institutions in determining FDI flow it needs to establish a relationship between determinants FDI frameworks and investment incentives framework. One of methods is dunning eclectic paradigm or OLI system relying on North approach or the impact of institutions on investment and economic activity. Dunning paradigm originally developed to describe the behavior of multinational corporations (MNEs) or how to companies benefit from foreign production facilities. Recently this paradigm mostly uses to analyze the determinants of FDI inflows (Gastanga & Nugent et al., 1998). It should be noted that the North approach about institutions mainly focused on their effects on economic activity and investment but it can also be used in the discussion of foreign direct investment. (Fathi A. Ali et.al.)

Eclectic paradigm and determinants factors of FDI inflows:

According to the dunning paradigm (1993) and (2001) any companies should have following three conditions to being a multinational organization: (1) having a special property that makes it distinctive compared to other firms in the host country. The assets may be tangible assets such as patents or products with intangible assets such as management skills, marketing or entrepreneurship. Dunning called such asset "ownership advantage" or "O advantage". (2) If the condition No. 1 achieves for the company, using the ownership advantages will be very useful through FDI and keeping these benefits in the form of local ownership or lease of these assets in order to prevent copying by rival companies. This advantage is called internal or internalization advantage or "I advantage". These advantages include an attempt to avoid the search and negotiation costs, moral hazard, and self-protection against non-compliance with contract terms. (3) In the host country, combining the ownership and internalization advantages with some locational advantages (L advantages) must be profitable for the company such as reducing input costs, the emergence of large and growing market, and so on. The third condition can explain the FDI distribution in most countries because it can be a specific advantage to a country. Dunning's paradigm suggests locational advantages which can be helpful in some countries attraction compared to other countries in the establishment of multinationals organizations (MNEs). These advantages may include some variables such as the availability of natural resources, price and quality, the quality of infrastructures, investment incentives, and economic systems and other strategies (Dunning (1993) and (1998)). Dunning (2001) argued that a variable such as locational advantage must be based on the assumption that companies are looking for places settlement of their activities in the situation with the highest profitability. In general, each variable would be likely to affect the profitability of a company's decisions concerning the location of investments can be considered in the set of variables that can affect the distribution of FDI in the countries. Dunning eclectic paradigm is a flexible tool for analyzing the determinants of FDI and any hypotheses about the determinants of FDI can be examined by Dunning eclectic paradigm.





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North argues that institutions have dominant effect on economic activities. Because they have dominant effect on transactions cost. Transaction costs include; (1) The cost of measuring the value of what is exchanged, (2) The costs related to rights protection, the policies and actions of the agreement. These costs are obtained from the opponent's behavior in economic exchanges due to lack of adequate knowledge. Uncertainty will be in the behavior of parties in economic transaction without such institutions. If the interests of each party is required, it may be a crook, disengagement agreements or shirk on their contract. Therefore, the concept of risk premium is considered on transactions costs. Both formal and informal institutions are explained in order to establish rules and guidelines to reduce uncertainty in economic transactions. Informal institutions such as the moral law (professional), behavioral norms and customs can reduce the level of uncertainty in transactions. Impersonal exchanges that allow fraud and the difference is high, need a third party to enforce agreements and reduce uncertainties between the parties. Governments can impose administrative mechanism to play the role of third party in transactions through investments law, effective justice system and other formal institutions. Therefore, if the parties' property rights do not defend and the contracts' implementation was difficult, the risk premium should be high. (Fathi A. et.al)

Institutions can be numerous possible effects on FDI inflows. Consequently, those countries which have proper institutional systems able to attract more foreign direct investment. Also recent developments in the global economy have changed the multinational organizations approach to the locational advantages. These organizations tend to have the best economic and institutional conditions (Dunning, 1998). These organizations do not attention to the countries with advantages in labor cost, availability of natural resources and etc. Rather, they seek advantages include a country's assets of knowledge, infrastructure and institutions. (Narula and Dunning, 2000)

Background research and empirical studies

Various research have been carried out on the relationship between the effective factors on FDI flow as well as the experience of some countries in relation with FDI inflows as follows: Table-1.1

Model, estimation method, variables and estimation results

Challenges in empirical study of the impact of institutions:

Experimental test about the impact of institutions on FDI faces some problems that make the test difficult. The important issue is the defining appropriate indicators for institutions. Determining these indicators is the important issues, especially with regard to the econometric methods. Another major challenge is the endogenous variables that if it does not solve the accurate estimation model will have bias. Because maybe foreign direct investment leads to the creation of effective institutions in the country. The fact that it is possible to increase investment and improves the quality of institutions, makes the institutional quality endogenous, creates problems in measurement error, reverses causation and creates a false correlation. This problem should solve by using a suitable econometric approach (Rigobon & Rodrik, 2005). High correlation between structural parameters and other control variables in this study as well as the size of government are some problems that cause multi linearity in the model.

Model and econometric method:

According to Brendan (2007) Bass and Hefeker (2005), Jensen (2003) and Lee & Resnik (2003) studies can use the following model to empirically examine the role of institutions that play in determining FDI:

$$FDI_{it} = \alpha_i + \beta Inst'_{it} + \gamma v'_{it} \delta + u_{it}$$

$$u_{it} = \varepsilon_{it} + v_{it}$$





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Where FDI_{it} indicates the dependent variable, $Inst$ indicates the vector of institutional variables (independent variables) include economic freedom, economic security, responsibility and right of the comment, CV indicates the vector of control variable (economic variables affecting on FDI) which is obtained from theoretical basis of the existing empirical determinants of FDI, α_i indicates component of the fixed effects (Fixed-effect method).

The suitable econometric methods for solving or reducing the problem of endogenous institutional indicators and correlation between institutional variables and other explanatory variables is the model estimating by generalized method of moments (GMM) of dynamic panel data. Econometric method which has been used to solve this problem is the econometric two-stage least squares method (2SLS). To use this method we should find a suitable variable to solve endogenous problem of institutional variables. However, this method has limits such as the difficulty of finding appropriate instrumental variables and these variables are limited. Moreover, this method cannot solve the correlation problem between explanatory variables and reduce or eliminate the linearity in the model. Caselli, et al (1996) estimated economic growth models by GMM techniques of dynamic panel data. Sachs (2003) said that determination of per capita income must be done with dynamic models. Bond, et al (2001) have investigated the use of this method in estimating growth models. Using GMM of dynamic panel data method have some advantages such as personal dissonance and remove the cross-sectional regressions. Therefore more accurate estimates with higher efficiency and less linear in the GMM have been achieved. GMM dynamic panel data method is used when the number of cross-cutting variables (N) is higher than the number of times (T) and years ($N > T$). It means that the number of countries is greater than the number of times (Bond, 2002; Baltagi, 2008).

Therefore the model as the main basis for assessing the role of institutions in determining FDI is used as follows:

$$FDI_{it} = \alpha_i + \beta_1 Freedom_{it} + \beta_2 Security_{it} + \beta_3 Voice_{it} + \delta_1 FDI_{it-1} + \delta_2 Exdebt_{it} + \delta_3 inflation_{it} + \delta_4 GCF_{it} + \delta_5 Trade_{it} + \delta_6 GDP_{it} + \delta_7 Nettax_{it} + \delta_8 Ggce_{it} + u_{it}$$

Research hypotheses:

- 1 - Institutional factor of economic freedom have dominant effect in attracting the FDI in developing countries.
- 2 - Institutional factor of voice and accountability have dominant effect in attracting the FDI in developing countries.
- 3 - Institutional factor of in economic security have dominant effect in attracting the FDI in developing countries.

Model variables

Control variables:

Economic variables (mentioned in the empirical literature), affecting on FDI were used in this study include: foreign debt, inflation rate (reflecting economic instability), gross fixed capital formation, trade (indicating the degree of economic openness), GDP (reflecting the size of economy), net of taxes, and ultimately the percentage of government consumption expenditures to GDP (as the size of government).

Independent variables:

In this study some institutional factors such as economic freedom, economic security, voice and accountability are introduced as independent variables.

The aspects of proposed institutional environment in this study is defined as follows:



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Economic Freedom: According to this research, economic freedom is one of the most important determinants in increasing the competition, investment, entrepreneurship, managers accountability and reducing financial corruption, rent-seeking and reducing the informal sector. One of the most important references for identification the economic situation in countries is the “Fraser Institution” which calculated the annual economic freedom index. The index data is derived from the World Bank WDI site. It includes five categories. (1) The size of government, expenditure, taxes and government participation. (2) Legal structure and protection of private property. (3) Access to base money. (4) Freedom to exchange with neighbors (5) laws relating to credits, labor market and commodity (business).

Voice and Accountability: The World Bank defines good governance based on six features (Kaufman, et al 2006): voice and accountability, political instability and violence, government effectiveness: government efficiency in duties, financial regulation: regulation and extra costs, the rule of law and control of corruption.

In this paper, the voice and accountability has considered as one the good governance indices. Voice and accountability as defined by the World Bank means that people be able to ask question against the government. (Including measures of civil and political freedom and freedom of the press), data for this indicator is derived from the World Bank WDI site.

Economic Security (capital risk): Economic security is a situation in which their all units pay attention to long-term planning without any fear of environmental hazards. In other words, creating the economic security means the creating a legal, social and political environment which the investment projects and economic activities can began without disturbing and external disturbances. Thus, in an economy where future conditions are somewhat predictable the economic security will be established. Also all data for this index is derived from the World Bank WDI site.

The results of the variables analysis and the results of the model estimation is presented in the below. It should be noted that the software Eviews8 has been used in order to test and model estimation.

Checking the Variables’ Stationary:

The presence or absence of unit root in the model variables should be investigated before the model estimation. It helps the results from model estimation not false and we will obtain more reliable results. When a stochastic process is stationary that the mean and variance are constant over time and the value of covariance between two time periods depends only on the distance or interval between two rounds and does have any communication to the real time of covariance. Generally the stationary test is one of the most important tests to estimating regression with reliable coefficient. Stationary test is used to avoid creating spurious regression. There are various tests are used in determining the stationary of panel data such as Levine Line, Fisher, and Im-Pesaran and Shin tests. In the present study some tests have been used such as adjusted Dickey Fuller-Fisher and the results are shown in table1.

According to the results in stationary tests of adjusted Dickey Fuller-Fisher , all studied variables except TAX and GDP are significant and H_0 can be rejected. Thus, all model variables except TAX and GDP are stationary by one subtract stage.

However, we must perform Kao Co-integration test whether the long-term relationships is confirmed or not?

The results in table 2 indicate a long-term relationship between variables and all the variables have a convergence over time.



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Period SUR and GMM method of dynamic panel data are used for model estimation. This test was performed for 114 developing countries with high, medium and low degree of human development in 2004 to 2011. According to the results in table 3, the effect of institutional factors along with economic factors has been investigated on foreign direct investment.

The results show in countries with a high human development (including 40 countries) that economic freedom index with 27.17 has the most impact, voice and accountability with 4.37, and eventually economic security with 3.32 has the least impact. Therefore they have dominant effect on foreign direct investment and significant at the 99% confidence level. In other words, for every one percent change in the economic freedom and voice and accountability indices the foreign direct investment changes 27 and 4.4 percent, respectively. Finally, for every ten percent change in the economic security index with one-year delay, the FDI changes 3.3 percent in the same direction.

According to economic variables the interrupted FDI with 0.072, foreign debt with – 0.73, inflation rate with 0.127, gross fixed capital formation with 0.273, trade (openness of economy) with 1.439, GDP (the size of economy) with 1.067, government's tax with – 1.14 and the ratio of government expenditure to GDP (reflecting the size of government) with 86 have been effective to attract foreign direct investment in these countries. All variables are significant at 99% level except the size of government. The ratio of government expenditure to GDP (size of government) is significant at 95% level. The direction of inflation impact and the size of government have been estimated unexpectedly.

The results show in countries with a medium human development (including 35 countries) that economic freedom index with 3.52 , voice and accountability with -1.33 , and eventually economic security with -0.434 has the least impact. Therefore they have dominant effect on foreign direct investment and significant at the 99% confidence level. In other words, for every one percent change in the economic freedom and voice and accountability indices the foreign direct investment changes 3.5 and -1.33 percent, respectively. Finally, for every ten percent change in the economic security index with one-year delay, the FDI changes -4.3 percent .

According to economic variables the interrupted FDI with 0.239 , foreign debt with – 0.644, inflation rate with -0.684 , gross fixed capital formation with -0.368 , trade (openness of economy) with 2.0138 , GDP (the size of economy) with 4.887, government's tax with – 2.20 and the ratio of government expenditure to GDP (reflecting the size of government) with 8.095 have been effective to attract foreign direct investment in these countries. All variables are significant at 99% level except the size of government. The ratio of government expenditure to GDP (size of government) is nonsignificant. The direction of size of government have been estimated unexpectedly.

The results show in countries with a Low human development (including 39 countries) that economic freedom index with -5.50 , voice and accountability with 0.187 , and eventually economic security with -0.011 has the least impact. Therefore they have dominant effect on foreign direct investment and significant at the 99% confidence level except the economic security. In other words, for every one percent change in the economic freedom and voice and accountability indices the foreign direct investment changes -5.5 and 0.19 percent, respectively. Finally, for every ten percent change in the economic security index with one-year delay, the FDI changes -0.11 percent .

According to economic variables the interrupted FDI with 0.2486 , foreign debt with -1.521, inflation rate with -0.353, gross fixed capital formation with 0.471, trade (openness of economy) with 5.071, GDP (the size of economy) with -2.54, government's tax with -0.51 and the ratio of government expenditure to GDP (reflecting the size of government) with 0.0033 have been effective to attract foreign direct investment in these countries. All variables are significant at



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99% level. The ratio of inflation rate and economic security index is significant at 95% level. The direction of inflation impact and the size of government have been estimated unexpectedly.

CONCLUSION

Although, the level of foreign direct investment has grown in the global level since the decade of the 1980s, but the share of many countries especially developing countries, was not significant. Institutional studies indicate that many institutions and institutional factors do not present in developing countries and if these factors were available they won't efficient. In the absence of appropriate institutions, motivations don't have dominant impact. Thus all markets need the supporting of non-market institutions. In this study, due to the inability of some countries to attract FDI, the factors affecting on FDI are divided into two categories; Economic and Institutional factors. There are strong reasons to attract more foreign direct investment in countries with strong institutions (including effective bureaucracy, low administrative corruption, lawful, proper implementation of contracts, and etc.) which is mentioned in the theoretical and experimental studies in the literature review.

According to the third paradigm of Dunning, those countries with strong institutions able to provide a locational advantage to attract foreign direct investment. In such cases, foreign investors get higher profitability and lower risk. Consequently, those countries which have proper institutional system able to attract more foreign direct investment.

Finding appropriate indicators for evaluating the impact of institutions on economic performance and institutional endogenous indices are some fundamental problems to evaluate the impact of institutions on economic performance. Economic freedom, voice and accountability and economic security in 2004-2011 are used in this paper as institutional indicators for developing countries with high, medium and low level of human development. All data were extracted from the World Bank website. The results showed significant impact of institutional factors (in addition to economic factors) to attract foreign direct investment. This shows that all countries, especially developing countries should perform institutional reform to attract more foreign investment in various sectors.

Empirical research related to institutions is just at the beginning stage and many questions exist in this area that should be addressed. Research on the effective institutions to attract foreign direct investment and degree of importance of each such matter is appropriate field for future studies.

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Appendix:

List of Countries (High HDI)

Argentina	Brazil	Jamaica	Macedonia	Saudi Arabia
Armenia	Chile	Jordan	Montenegro	Serbia
Azerbaijan	Colombia	Kazakhstan	Mauritius	Tonga
Bulgaria	Algeria	Kuwait	Malaysia	Tunisia
Bahamas	Ecuador	Libya	Panama	Turkey
Bosnia	Georgia	Lithuania	Poland	Ukraine
Belarus	Croatia	Latvia	Romania	Uruguay
Belize	Iran	Mexico	Russian	Venezuela

List of Countries (Medium HDI)

Bolivia	Gabon	Cambodia	Nicaragua	Syrian Arab Republic
Botswana	Guatemala	Lao PDR	Pakistan	Thailand
China	Guyana	Sri Lanka	Philippines	Tajikistan
Congo, Rep.	Honduras	Morocco	Paraguay	Turkmenistan
Dominican Republic	Indonesia	Maldives	El Salvador	Timor-Leste
Egypt, Arab Rep.	India	Mongolia	Suriname	Uzbekistan
Fiji	Kyrgyz Republic	Namibia	Swaziland	Vietnam

List of Countries (Low HDI)

Afghanistan	Comoros	Kenya	Malawi	Sierra Leone
Angola	Djibouti	Liberia	Niger	Chad
Burundi	Ethiopia	Lesotho	Nigeria	Tanzania
Benin	Ghana	Madagascar	Nepal	Uganda
Burkina Faso	Guinea	Mali	Papua New Guinea	Yemen, Rep.
Bangladesh	Gambia, The	Myanmar	Rwanda	Zambia
Cote d'Ivoire	Guinea-Bissau	Mozambique	Sudan	Zimbabwe
Cameroon	Haiti	Mauritania	Senegal	





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Table-1.1:

Rank	Author	Year	Title	Results
1	Jude, Cristina	2013	Growth effect of FDI In developing economies: The role of institutional quality	Improving in the institutional framework must precede to the FDI attraction to impact on growth.
2	Blanton. R	2012	Rights, Institutions, and Foreign Direct Investment: An Empirical Assessment, Foreign Policy analysis	Domestic institutions, social rights and participation in international institutions and trade agreements are the effective factors on the foreign direct investment flow.
3	Holmes, R.M.et.al	2011	The Interrelationships among Informal Institution, Formal Institutions, and Inward Foreign Direct Investment	Formal institutions of a country (set of political-regulatory and the future directions for economic institutions) and culture as informal representative institutions are effective in attracting foreign direct investment.
4	Bissoon	2011	Can Better Institutions After More Foreign Direct Investment (FDI)?	Control of corruption, rule of law, political stability and freedom of media, as institutional quality indices are effective on FDI. The institutional factors are complementary and their combined effects have more effect on FDI.
5	Fathi A. Ahmed Ali	2010	Essays on foreign Direct Investment, Institution and Economic Growth	The relationship between property rights (as representative of institutional quality) and FDI and then their relationship with economic growth
6	Kohler, philippe	2010	Foreign direct investment in countries with weak institution	Weak institutions are a barrier for foreign direct investment. Centralization of corruption and administrative bureaucracy increases the cost of multinational international firms and have negative effect on their incentives to invest.
7	Wernick, A. D., J. Haar, and S. Shane	2009	Do Governing Institutions Affect Foreign Direct Investment Inflows?	The quality of regional institutions is an important factor in explaining the FDI differences in the countries. The positive effects of good regional governance on FDI is more than the impact of developed regional market.
8	Dumludag, Devrim & Sukruoglu, Deniz	2007	The Impact of Macroeconomic and Institutional Variables on Foreign Direct Investment Flows in Emerging Markets,	The main reason for the uneven distribution of FDI in developing countries is the difference in institutional quality (political and economic stability, civil rights, corruption and crises) between different countries.
9	Daude. CH & Stein. E	2007	The quality of Institutions and foreign Direct Investment	Better institutional quality has a significant positive economic impact on foreign direct investment. Particularly laws, regulations and regulatory policies in contrast with the





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				political instability and lack of commitment in the contracts have an important role in preventing the FDI attraction.
10	Jakobsen J., and I. de Soysa	2006	Do Foreign Investors Punish Democracy?	In addition to the effect of democracy on protection of property rights, the establishment of democracy by increasing the flow of free trade and its impact on the educational level of workforce will increase the growth of foreign direct investment inflows.
11	Asiedu, E	2005	Foreign Direct Investment in Africa	Unlike his previous study (2002) concluded that less administrative corruption, political stability and reliable system of law can attract the FDI.
12	Globerman, S. ; D. Shapiro and Y. Tang	2004	Governance and foreign Direct Investment in Emerging and Transition European Countries	Transition and emerging countries in Europe (due to issues related to governance) have provided better conditions than other developing countries to attract FDI in the world.
13	Harms, P.and H.ursprung	2003	Do civil and political repression really boost foreign direct investment?	FDI will be seen in countries where there is civil liberties and political freedoms and they are respected.
14	Globerman, S and D. Shapiro	2002	Global Foreign Direct Investment Flows: The Role of Governance Infrastructure	Investment in governance structure has a lot of effect on FDI on the one hand with the attraction of foreign capital and on the other hand, with the rise of multinational companies and their investment in abroad.
15	Busu. A, and Srinvasan. K,	2002	Foreign Direct Investment in Africa	African efforts to establish political and economic stability in the macro level and structural reforms in key sectors make some African countries success in attracting FDI.

Table1 - Measuring unit root in variables

The title of variable	The test statistic ADF – Fisher countries with high degree of human development		The test statistic ADF – Fisher countries with medium degree of human development		The test statistic ADF – Fisher countries with low degree of human development	
	Statistic value	Recognition	Statistic value	Recognition	Statistic value	Recognition
FDI	163.652 (0.4052)	All Level I(0)	32.61 (0.000)	All Level I(0)	79.06 (0.4452)	All Level I(0)





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EFCI	142.833 (0.0000)	All Level I(0)	71.36 (0.0253)	All Level I(0)	84.30 (0.0313)	All Level I(0)
ES	118.511 (0.0034)	All Level I(0)	88.99 (0.0624)	All Level I(0)	95.30 (0.0890)	All Level I(0)
VA	100.54 (0.0601)	All Level I(0)	134.19 (0.000)	All Level I(0)	78.05 (0.4771)	All Level I(0)
EXDEBT	64.69 (0.5911)	All Level I(0)	64.39 (0.4628)	All Level I(0)	57.32 (0.9242)	All Level I(0)
INFLATIONCP	217.65 (0.0000)	All Level I(0)	100.54 (0.0024)	All Level I(0)	165.74 (0.000)	All Level I(0)
GCF	201.18 (0.0000)	All Level I(0)	145.97 (0.000)	All Level I(0)	115.45 (0.000)	All Level I(0)
TRADE	90.42 (0.1997)	All Level I(0)	72.30 (0.3378)	All Level I(0)	85.85 (0.2541)	All Level I(0)
GDP	16.28 (1.0000)	All Level I(1)	7.13 (1.000)	All Level I(1)	7.69 (1.000)	All Level I(1)
TAX	31.38 (1.0000)	All Level I(1)	25.78 (0.9999)	All Level I(1)	39.30 (0.9731)	All Level I(1)
GGCE	93.02 (0.1514)	All Level I(0)	61.56 (0.6960)	All Level I(0)	70.35 (0.5987)	All Level I(0)

Source: Research findings

Table2 - Kao Residual Co-integration Test

	Prob.	t-statistic
ADF (High)	0.0202	2.4895
ADF (Medium)	0.0224	-0.4611
ADF (Low)	0.1129	1.2112

Source: Research findings

Table3. Results of model estimation by GMM method

Dependent Variable LOG FDI	HIGH		MEDIUM		LOW	
	Coefficients	Prob.	Coefficients	Prob.	Coefficients	Prob.
C (Fixed effect)	-41.63	0.0000	-94.55	0.0000	103.08	0.0000
LOG FDI(-1)	0.072	0.0036	0.2391	0.0053	0.2486	0.0001
LOG EXDEBT	-0.730	0.0000	-0.644	0.2476	-1.521	0.0000
LOG INFLATIONCP	0.1275	0.0037	-0.684	0.0001	-0.353	0.0130





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LOG GCF	0.2736	0.000	-0.368	0.0075	0.471	0.0002
LOG TRADE	1.4394	0.0015	2.0138	0.0016	5.071	0.0000
LOG GDP	1.0675	0.0065	4.8875	0.0002	-2.54	0.0003
LOG TAX	-1.142	0.0021	-2.203	0.0002	-0.51	0.0025
LOG GGCE	0.8632	0.0342	8.095	0.0033	0.0033	0.0000
LOG ES	0.3317	0.0002	-0.434	0.0089	-0.011	0.0190
LOG VA	4.3697	0.0000	-1.330	0.0001	0.1876	0.0013
LOG EFCI	27.174	0.0000	3.5196	0.0007	-5.501	0.0003
J-Statistic	8.8811	0.3524	0.4163	0.9987	5	0.4159

Source: Research findings





RESEARCH ARTICLE

Diagnostic Efficacy of Radiography and Sonography in Detection of Knee Osteoarthritis

Hadi Majidi¹, Fatemeh Niksolat^{2*} and Khatereh Anbari³

¹Assistant Professor of Radiology, Mazandaran University of Medical Sciences, Sari, Iran

²Assistant Professor of Rheumatology, Mazandaran University of Medical Sciences, Sari, Iran.

³Assistant Professor of Community Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.

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*Address for correspondence

Fatemeh Niksolat

Assistant Professor of Rheumatology,

Mazandaran University of Medical Sciences,

Sari, Iran.

E-mail: fniksolat@yahoo.com.



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ABSTRACT

Objective: Knee osteoarthritis is the most common degenerative disorder occurring in elderly people. Radiography and sonography are convenient techniques to detect diverse pathological features of knee osteoarthritis. These imaging tests are non-expensive and routine techniques compared to expensive methods such as magnetic resonance imaging. The aims of present study was to evaluate diagnostic efficacy of radiography and sonography in detection diverse features of knee osteoarthritis.

Method: Fifty patients (40 women and 10 men, mean age 41.2 ± 6.1 years) with clinical and radiographic diagnosis of knee osteoarthritis referred to the Rheumatology Clinic of the Shohada Hospital of Khorramabad were evaluated during May 2012 until May 2013. All obtained radiographic and sonography images were evaluated by two expert blinded rheumatologist and the sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and accuracy with 95% confidence intervals were analyzed.

Results: Prevalence of the marginal osteophyte, Geod and decreased cartilage thickness were significantly increased in \square 40 patients compared to \leq 40 participants (all $P \square 0.001$). The incidence of diverse features of osteoarthritis were not significantly changed regarding to patient's gender except decreased cartilage space based on MRI data. Specificity of radiography was superior to sensitivity.





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Conclusion: In patients with different features of osteoarthritis, radiography is a reliable and specific imaging modality. Sonography is a reliable imaging test and more sensitive in detection of decreased cartilage thickness. Radiography is specific test to detect of subchondral sclerosis and is reliable in positive cases.

Key words: Knee osteoarthritis; Sensitivity; specificity; Radiography; Ultrasound,

INTRODUCTION

Osteoarthritis of the knee joint as a degenerative disorder is the most common diagnosis arthropathy cause of pain and disability in older patients(1). The prevalence of osteoarthritis in adult individuals increases with age(2). Other risk factors include gender, overweight, knee injury and family history. About %25 of population older than 55 years show signs of knee osteoarthritis in radiography(3). However, osteoarthritis shows variability in pathological evidence and clinical symptoms respect to various parameters such as deposition of calcium crystal and presence of inflammation(4).

There is no specific methods for diagnosis of clinical osteoarthritis. Currently, radiography is usually used as initial imaging test in patients carrying knee osteoarthritis and to assess the severity of the disease(5). Radiographic features of osteoarthritis include joint space, marginal osteophytes and subchondral sclerosis, showing pathological changes(6).

Sonography is reliable and valid test to elucidate several features of knee osteoarthritis uses routinely in rheumatology clinics. Ultrasound provides data about thickness and integrity of cartilage. Also, ultrasound has some advantages over magnetic resonance imaging (MRI) includes low cost, easier to use, convenient and involves no radiation(7).

Although radiography and sonography is commonly used to evaluate patients with knee osteoarthritis, the usefulness of mentioned imaging test to detect several features of osteoarthritis have documented in few previous studies. Thus, the aim of the current study was to evaluate the prevalence of diverse features of knee osteoarthritis in patients referred to rheumatology clinics using magnetic resonance imaging, sonography and radiography and determination of diagnostic efficacy indices consists specificity, sensitivity, positive predictive and negative predictive value and accuracy of radiography and sonography to diagnosis diverse features of knee osteoarthritis in comparison to MRI as gold standard test.

MATERIALS AND METHODS

Patients and approaches

In present study, fifty consecutive patients (40 women and 10 men, age mean 41.2 ± 6.1) with osteoarthritis referring to the Rheumatology Clinic of the Shohada Hospital of Khorramabad were evaluated during May 2012 until May 2013. The inclusion criteria was to have the radiographic imaging of knee OA after verification of expert rheumatologist for presence of osteoarthritis, the radiography, sonography and MRI imaging were taken according to previous studies(8) and the images were analyzed by two expert blinded practitioners. The diagnosis of knee OA was established according to the American College of Rheumatology criteria. The study was conducted according to the Helsinki Declaration and written consent was obtained from all participants.



**Fatemeh Niksolat et al.****Statistical analysis**

The mean \pm standard deviation (SD), median and other needed values were calculated. For evaluating the diagnostic performance of radiography and sonography examinations in comparison to MRI as gold standard test the sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and accuracy with 95% confidence intervals were analyzed using X^2 test by SPSS software version 16.00. For all statistical outputs, p value 0.05 was accepted.

RESULTS**Evaluation of osteoarthritis in knee using MRI:**

Based on MRI findings for knee osteoarthritis, the marginal osteophyte, Geod and decreased cartilage thickness were significantly increased in \square 40 patients compared to \leq 40 participants (all $P \square 0.001$). The MRI information of patients are presented in Table 1 with details. In contrast, the prevalence of knee osteoarthritis were not remarkably changed based on gender of patients except decreased cartilage space that was significantly enhanced in men compared to women (Table 2).

Evaluation of osteoarthritis in knee using ultrasound imaging

The sonography was performed for all selected patients and data revealed that marginal osteophyte and decreased cartilage thickness were significantly augmented in older patients ($P=0.001$ and $P \square 0.001$ respectively) (Table 3). The prevalence of osteoarthritis's different features were not affected by gender of participants (Table 4).

Evaluation of osteoarthritis in knee using radiography

The prevalence of diverse features of knee osteoarthritis are fully given in Table 5. According to obtained data, the prevalence of marginal osteophyte, Geod and subchondral sclerosis were noticeably increased in patients older than forty years old in comparison to patients having less than forty years old. In parallel with obtained information of ultrasound imaging, the features of knee osteoarthritis were not significantly changed by gender in radiographs images (Table 6).

Diagnostic efficacy indices of radiography and ultrasound imaging

Sensitivity and specificity, PPV and NPV regarding radiography and sonography were measured and presented in Table 7. Based on analyzed data, specificity and positive predictive value of radiograph imaging were greater than sensitivity and negative predictive value in all types of knee osteoarthritis. The sensitivity of radiography to detect Geod and subchondral sclerosis was very low (33.3 and 23% respectively). Sonography information revealed that diagnostic performance indices for diverse types of knee osteoarthritis are different (Table 8). Same to radiographs images, the specificity of sonography to diagnosis of marginal osteophytes was higher than its sensitivity. In contrast, sensitivity of sonography examination for detection of cartilage thickness was more reliable compared to its specificity (90.4% against 75.8%).

DISCUSSION

According to our data regarding MRI and ultrasound imaging, the incidence of decreased cartilage thickness significantly augmented in patients older than 40 years old. Conversely, some previous studies showed age of participant were not significantly associated with the incidence of decreased cartilage thickness (9, 10). Also, decreased cartilage thickness and marginal osteophytes were most common pathological features in patients having knee osteoarthritis. In parallel to our data, Kornatp et al reported that cartilage thickness and marginal osteophytes are common features of knee osteoarthritis based on MRI data (4).



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Despite development of more sensitive and specific imaging techniques, the radiography remains most common diagnosis test in rheumatology clinics to detect osteoarthritis of knee. Diagnostic efficacy of radiography and sonography were analyzed compared to MRI information. Obtained data indicated that specificity and positive predictive value of radiography is more reliable than its sensitivity and negative predictive value. Thus, Positive reports of radiography imaging are more trustworthy compared to negative reports. Also, Data showed that the subchondral features of knee osteoarthritis have properly diagnosed using radiography and MRI imaging as a non-convenient and expensive technique is not needed.

About sonography imaging, data showed that sensitivity and specificity of sonography are changed regarding diverse features of knee osteoarthritis. But, ultrasound showed great agreement with MRI obtained data in overall. Specificity of sonography in detection of marginal osteophyte is more than its sensitivity (91.3 against 74). In contrast, sonography is more sensitive to diagnosis of decreased cartilage thickness. Other studies also reported that sonography is specific to detect of marginal osteophyte (11-13). Also, Many studies showed that sonography is sensitive to diagnosis of decreased cartilage thickness (14).

Conversely previous data showed all cartilage effusion were detected using ultrasound imaging. But, according to presented data, just %70.5 of cases having cartilage effusion have diagnosed by ultrasound imaging (13). This controversial data may be due to different operator, diverse instruments various sample size and different interpretation by rheumatologist. About medial and lateral meniscus rupture, sonography was not reliable imaging examination in comparison to MRI according to our data. Although, Lee et al 1996 reported that sensitivity of sonography to detect of Medial collateral ligament (MCL) injury is 94% (15). Other researches also indicate that sonography is a very operator dependent modality imaging (11).

In conclusion, radiography and sonography as two routine imaging techniques in the detection of knee osteoarthritis are useful especially to diagnosis the positive cases. Specificity of radiography is superior to sensitivity for all pathological features of osteoarthritis. Sensitivity of sonography imaging to detect of some features of knee osteoarthritis such as decreased cartilage thickness is superior to radiography.

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Table 1: Detection of different features of osteoarthritis in knee using MRI according to patient's old

Type of pathology	Yes	NO	Total	P value
Decrease cartilage space				
≤ 40	6 (20.7)	23 (79.3)	29 (100)	0.001
□ 40	9 (42.9)	12 (57.1)	21 (100)	
Marginal Osteophyte				
≤ 40	10 (34.5)	19 (65.5)	29 (100)	0.001
□ 40	17 (81)	4 (19)	21 (100)	
Geod				
≤ 40	2 (6.9)	27 (93.1)	29 (100)	0.025
□ 40	7 (33.3)	14 (66.7)	21 (100)	
Cartilage Thickness				
≤ 40	6 (20.7)	23 (79.3)	29 (100)	□0.0001
□ 40	15 (71.4)	6 (28.6)	21 (100)	
Cartilage effusion				
≤ 40	9 (31)	20 (69)	29 (100)	0.6
□ 40	8 (38.1)	13 (61.9)	21 (100)	
Medial Meniscus rupture				
≤ 40	14 (48.3)	15 (51.7)	29 (100)	0.34
□ 40	13 (61.9)	8 (38.1)	21 (100)	
Lateral Meniscus rupture				
≤ 40	2 (6.9)	27 (93.1)	29 (100)	0.5
□ 40	0 (0)	21 (100)	21 (100)	
subchondral sclerosis				





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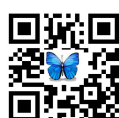
≤ 40	2 (6.9)	27 (93.1)	29 (100)	0.63
□ 40	3 (14.3)	18 (85.7)	21 (100)	

Table 2: Detection of different features of osteoarthritis in knee using MRI according to patient's gender

Type of pathology	Yes	NO	Total	P value
Decrease cartilage space				
Women	9 (22.5)	31 (77.5)	40 (100)	0.048
Men	6 (60)	4 (40)	10 (100)	
Marginal Osteophyte				
Women	23 (57.5)	17 (42.5)	40 (100)	0.48
Men	4 (40)	6 (60)	10 (100)	
Geod				
Women	6 (15)	34 (85)	40 (100)	0.35
Men	3 (30)	7 (70)	10 (100)	
Cartilage Thickness				
Women	17 (42.5)	23 (57.5)	40 (100)	0.88
Men	4 (40)	6 (60)	10 (100)	
Cartilage effusion				
Women	13 (32.5)	27 (67.5)	40 (100)	0.71
Men	4 (40)	6 (60)	10 (100)	
Medial Meniscus rupture				
Women	21 (52.5)	19 (47.5)	40 (100)	0.73
Men	6 (60)	4 (40)	10 (100)	
Lateral Meniscus rupture				
Women	1 (2.5)	39 (97.5)	40 (100)	0.27
Men	1 (10)	9 (90)	10 (100)	
subchondral sclerosis				
Women	3 (7.5)	37 (92.5)	40 (100)	0.25
Men	2 (20)	8 (80)	10 (100)	

Table 3: Detection of different features of osteoarthritis in knee using sonography according to patient's old

Type of pathology	Yes	NO	Total	P value
Marginal				





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Osteophyte				
≤ 40	7 (24.1)	22 (75.9)	29 (100)	0.001
□ 40	15 (71.4)	6 (28.6)	21 (100)	
Cartilage Thickness				
≤ 40	9 (31)	20 (69)	29 (100)	□ 0.0001
□ 40	17 (81)	4 (19)	21 (100)	
Cartilage effusion				
≤ 40	10 (34.5)	19 (65.5)	29 (100)	0.54
□ 40	9 (42.9)	12 (57.1)	21 (100)	
Medial Meniscus rupture				
≤ 40	10 (34.5)	19 (65.5)	29 (100)	0.11
□ 40	12 (57.1)	9 (42.9)	21 (100)	
Lateral Meniscus rupture				
≤ 40	0 (0)	29 (100)	29 (100)	-
□ 40	0 (0)	21 (100)	21 (100)	

Table 4: Detection of different features of osteoarthritis in knee using sonography according to patient's gender

Type of pathology	Yes	NO	Total	P value
Marginal Osteophyte				
Women	18 (45)	22 (55)	40 (100)	0.39
Men	4 (40)	6 (60)	10 (100)	
Cartilage Thickness				
Women	22 (55)	18 (45)	40 (100)	0.77
Men	4 (40)	6 (60)	10 (100)	
Cartilage effusion				
Women	14 (35)	26 (65)	40 (100)	0.38
Men	5 (50)	5 (50)	10 (100)	
Medial Meniscus rupture				
Women	19 (47.5)	21 (52.5)	40 (100)	0.48
Men	3 (30)	7 (70)	10 (100)	
Lateral Meniscus rupture				
Women	0 (0)	40 (100)	40 (100)	-
Men	0 (0)	10 (100)	10 (100)	





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Table 5: Detection of different features of osteoarthritis in knee using radiography according to patient's old

Type of pathology	Yes	NO	Total	P value
Decrease cartilage space				
≤ 40	6 (2.7)	23 (97.3)	29 (100)	0.091
□ 40			21 (100)	
Marginal Osteophyte				
≤ 40	7 (24.1)	22 (75.9)	29 (100)	0.018
□ 40	12 (57.1)	9 (42.9)	21 (100)	
Geod				
≤ 40	0 (0)	29 (100)	29 (100)	0.14
□ 40	4 (19)	17 (81)	21 (100)	
subchondral sclerosis				
≤ 40	4 (13.8)	25 (86.2)	29 (100)	0.021
□ 40	9 (42.9)	12 (57.1)	21 (100)	

Table 6: Detection of different features of osteoarthritis in knee using radiography according to patient's gender

Type of pathology	Yes	NO	Total	P value
Decrease cartilage space				
Women	12 (30)	28 (70)	40 (100)	1
Men	3 (30)	7 (70)	10 (100)	
Marginal Osteophyte				
Women	14 (35)	26 (65)	40 (100)	0.47
Men	5 (50)	5 (50)	10 (100)	
Geod				
Women	3 (7.5)	37 (92.5)	40 (100)	0.79
Men	1 (10)	9 (90)	10 (100)	
subchondral sclerosis				
Women	10 (25)	30 (75)	40 (100)	0.7
Men	3 (30)	7 (70)	10 (100)	





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Table 7: Diagnostic efficacy indices for radiography in detecting different features of osteoarthritis

Radiography	Decrease cartilage space	Marginal osteophyte	Geod	Subchondral Sclerosis
	Percentage (95 % CI)	Percentage (95 % CI)	Percentage (95 % CI)	Percentage (95 % CI)
Sensitivity	73.3 (44.8-91)	66.6 (46-82.7)	33.3 (9 – 69)	23 (6.1-54.1)
Specificity	88.5 (72.3-96.2)	95.6 (76-99.7)	97.5 (85.5-99.8)	94.5 (80.4-99)
Positive predictive value	73.3 (44.8-91)	94.7 (71.8-99.7)	75 (21.9-98.6)	60 (17-92.7)
Negative predictive value	88.5 (72.3-96.2)	70.9 (51.7-85.1)	76.9 (73-94.5)	77.7 (62.5-88.2)
Accuracy	84	80	86	76

Table 8: Diagnostic efficacy indices for sonography in detecting different features of osteoarthritis

Sonography	Cartilage effusion	Marginal osteophyte	Cartilage thickness	Medial Meniscus rupture	Lateral Meniscus rupture
	Percentage (95 % CI)	Percentage (95 % CI)	Percentage (95 % CI)	Percentage (95 % CI)	Percentage (95 % CI)
Sensitivity	70.5 (44-88.6)	74 (53.4-88.1)	90.4 (68.1-98.3)	62.9 (42.4-79.9)	0 (0-80.2)
Specificity	78.7 (60.6-90.3)	91.3 (70.4-98.4)	75.8 (56-88.9)	78.2 (55.7-91.7)	1 (90.7-1)
Positive predictive value	63.1 (38.6-82.7)	90.9 (69.3-98.4)	73 (51.9-87.6)	77.2 (54.1-91.3)	-
Negative predictive value	83.8 (65.5-93.9)	75 (54.7-88.5)	91.6 (71.5-98.5)	64.2 (44.1-80.6)	96 (85.1-99.3)
Accuracy	76	82	82	70	96





RESEARCH ARTICLE

An Investigation on Zearalenone Toxin Contamination in Flourcrops from 11 Cities of I.R.Iran

Alizademanizhe*, Chaychi Nosrati Arash, Modiri Leila and lakzaieazar Omid

Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch , Islamic Azad University(IAU), Lahijan, Iran.

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*Address for correspondence

Alizademanizhe

Department of Microbiology, Faculty of Basic Sciences,
Lahijan Branch, Islamic Azad University (IAU),
Lahijan, Iran.

E-mail: alizade_manizhe@yahoo.com



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ABSTRACT

Mycotoxins are small, toxic chemical products produced as secondary metabolites by a few fungal species that readily colonise crops and contaminate them with toxins in the field or after harvest. In this study a liquid chromatographic method was developed for the simultaneous determination of aflatoxins (AFs) (B₁, B₂, G₁, and G₂), ochratoxin A (OTA) and zearalenone (ZEA) toxins in flour. This cross-sectional study was carried out in Spring - Summer. Zearalenone levels vary in different cities of Iran and are higher than national standard limits (200 µg/Kg per BW) and, therefore, according to the harvesting programmes for the wheat crop. Thus the Ijrud city with amount of 21.63ppb and Zanjan, Gonbad city of Golestan province, with the least amount of zearalenone by 14.9 ppb have been defined respectively. Considering these results, these special products should not be a health concern for these collectives; however, special attention needs on foods distribution in retail shops.

Key words: Zearalenone, Fusarium, flour, Cities, Iran

INTRODUCTION

Members of the mycotoxin-producing fungal species, Fusarium, occur as facultative saprophytes and parasites. They are widespread in nature, and frequently contaminate many crops (corn, wheat, rye, oats, and rice) by producing a wide range of toxins [Shier W.T et al., 1998]. The most hazardous Fusarium mycotoxins, in terms of animal health and productivity, are deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FBs) and moniliformin [D'Mello et al., 1999]. Toxins AFs (B₁, B₂, G₁ and G₂) are mycotoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus*.



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These mycotoxins are hepatotoxic and carcinogenic in humans. *A. flavus* produces AFB₁ and B₁, while *A. parasiticus* gives rise to AFB₁, B₁, G₁ and AFB₂ are also produced by some other *Aspergillus* species (*A. nomius*, *A. pseudotamarii*, *A. bombycis*), although they are not as important in economical terms as *A. flavus* and *A. parasiticus*. AFBs occur all over the world in foods and in a wide variety of food raw materials, most commonly in peanuts, pistachio, nuts, cereals, maize, rice and figs. Zearalenone has been shown to occur in almost every agricultural products and a variety of food-grade grains and foods have been found to contain this mycotoxin including corn and corn products, breakfast cereals, corn beer, wheat flour, bread and walnuts and in animal feed products. As zearalenone is metabolised by yeasts during brewing, mainly to be zearalenol, this metabolite should be looked for in beer. The presence of zearalenone in whole plants and parts of maize used for silage making was investigated in Germany during 1989-1990 and zearalenone was detected at concentrations up to 300 µg/kg mainly accumulating at the end of the ripening process, with subsequent contamination of the silage. Because zearalenone and its metabolites are produced by the same fungi that give rise to deoxynivalenol and nivalenol it is not surprising that mixtures of all these mycotoxins can occur in the same sample. It may be produced in wheat, barley, rice, maize, and other cereals and in some other food crops and can survive into consumer products. In cereals and animal feeds, closely related compounds or conjugated products are known to also occur. Some of these are considered to be more potent oestrogens than the parent compound. Because of these complex interactions some of these zearalenone-related chemicals may enter the food chain in animal products such as meat and milk. Attempts are now being made to determine the extent of this secondary source of contamination. This study was designed to determine the presence and levels ZEA in Wheat flour, that especially sold and consumed in Khorasan province of Iran and to compare the obtained results with maximum AFLs, OTA and ZEA tolerance limits that accepted by Iranian national standard.

MATERIALS AND METHODS

This cross-sectional study was carried out both in the field and in laboratory situations in Spring-Summer (March-August) 2013. New harvested and cropped flour samples obtained from early March-late August from 7 provinces with a high level productions in the state including the southern provinces (Khozestan), West (including Kermanshah, Hamedan) and North (Zanjan, Ardebil, Mazandaran, Golestan) a series per one hundred tone of each batch storages provided. After preparation, the flour specimens collected for use dried, and adjusted its humidity on 14%, mixing and re-mixing were done for each grouped samples, taken four sample groups of 100 Grams randomly selected as a sample in order to measure, control, storage and samples for grind in then meal or flour wheat samples taken longer phase of the laboratory mill followed by the flour samples were been ready for extraction. In this method, the number of specimens was taken from three parts different of each batch (both sides and middle of the batches). Next, by mixing these three parts, approximately 200 g was taken as a final sample and kept in a closed container in the refrigerator.

Preparation of Samples

The samples have been stored in a cool place; protected from light. A representative sample (according to accepted sampling techniques) have been ground and thoroughly mixed prior to proceeding with the extraction procedure. Weighing 5 g of ground sample and added it to a suitable container with 25 ml of methanol (70%), shaking vigorously for 3 min (manually with shaker), filtered the extract through Whatman No.1 filter, diluted 1 ml of the obtained filtrate with 1 ml of distilled or deionized water finally used 50 µl of the filtrate per well in the test sample size may be increased if required, but the volume of methanol/water must be adapted accordingly.

Test procedure

Inserting a sufficient number of wells into the micro well holder for all standards and samples to be run. Recorded standard and sample positions. Pipetted 50 µl of standard or prepared sample to separate well. Added 50 µl of enzyme conjugate (red cap) to the bottom of each well then 50 µl of the anti-T-2 toxin antibody solution to each well. Mix gently





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by shaking the plate manually and incubation for 10 min, at room temperature, dumping the liquid out of the wells into a sink. Taped the microwellholder upsidedown onto a clean filter towel to remove all remaining liquid from the wells. Using a multichannelpipette, filled the wells with deionized water (250µl per well) then Emptied the wells again and removeall remaining liquid.Repeatingthe washing step two more times. Added 100µl substrate/chromogentoeachwell. Mixed gently by shaking the plate manually and incubate for 5 min at room temperature in the dark. Adding 100µl of stop solution to each well. Mixing gently by shaking the plate manually and measured the absorbance at 450 nm to be Read within 10 minutes after addition of stop solution. For single determinations we recommend log it/log evaluation and for double or multiple determinationscubicspine should be used. The course of the standard curve is shown in the quality Assurance Certificateenclosed in the test kit.

$$\text{AbsorbanceStandard\%} = \frac{\text{Absorbance of standard}}{\text{Absorbance of NegCon}} \times 100$$

RESULTS AND DISCUSSION

According to the Table 1.1. T-toxin levels vary in different cities of Iran and are higher than national standard limits (200 µg/Kg per BW) of measuring the amount of toxins in the research evaluation unit ppb and , therefore, according to the charts in order to harvesting programms for the wheat crop from left to right , Khuzestan province State city ;Ahvaz (Ahv), 20.12 ppb, Gonbad city of Golestan province (Gon), 14.92ppb, the Kalaleh city of Golestan province (kal), 18.61 ppb, Ali Abad province 17.56 ppb, Neka , Mazandaran (Nek), 19.25ppb and the state City Sari, Mazandaran Province (Sar), 15.13ppb, Khodabandeh city, Zanjan Province (Kho), 16.12ppb, Ayjrud county city agricultural State Branch(ljr), 21.63 ppb Germmy city of Ardabil province (Ger), 18.35ppb and Meghann county city agricultural State Branch (Mog) 16.34and Ardabil province State city (Ard), 17.89,for the city of Razan (Raz) and famenin (Fam) for the Hamedan province were respectively 17.19ppb, 16.17ppb and finally the amount of quantified toxins for Kermanshah Province State city (KeS) was 31.2 ppb have the highest T-toxin poison into account . Thus, according to (Table1) the ljrud city with a amount of 21.63ppb and zanjan and Sari , Gonbad city of Golestan province, with the least amount of zearalenone by 14.9 ppb have been defined respectively [Table1].As we can see in table 2, in evaluation of different kinds of sampling methods and the amounts of sample, the distribution of the sampling sites and sample preparation and evaluation of the results and so on, Measurement and analysis of numerical values obtained according to the diagram, Skewness values for aflatoxin in wheat grain was 60%, aflatoxin in flour was 1.19%, wheat zearalenone was 76% and flour zearalenone was 54%. According to Kurtosis, aflatoxin in wheat was- 0.76, aflatoxin in flour was+ 5.5, wheat zearalenon -0.51 and flour zearalenone was +0.1. It is clear that the numerical data follow a norma distribution [Table 2].In examining how different figures obtained from measuring the amount of copper in wheat flour samples were shown in the diagram above, the maximum amount F Zea was in 16-19 ppb and the minimum amount obtained in 20-23 ppb[Figure 2]. Given the potential effects of toxic and carcinogenic mycotoxins, these are one of the most important legal issues of care and attention in the all countries. So that's why regular screenings for detect the broad and strict control of food and toxins are currently being done. This has led to serious restrictions on the types and the permissible limit of food poisoning [YazdanpanahH., 2006, Meng K., 2010]. Mycotoxin levels can be reduced by different techniques, for example through technical methods and agricultural chemical or physical methods during the course of cultivation of cereals could be reduced during maintenance. Chemical reduction of mycotoxins is significant and it consists of chemical protection of plants during the growing period using the most effective plant protection products (Miller R., 1995, Richard J.L., 2007). Also different kinds of procedures to dry grains before storing were used, like drying at high temperature, at low temperature, drying through sun light and etc, to decrease the levels of moisture in grains. Using biological methods like propanoic acid alone or with ascetic acid and formic acid were successfully control the contamination of grain by fungi. In this kind of methods, adding a few amounts of 8-quinolinol to food is highly reducing the amount of propionic acid [Miller R., 1995, Richard J.L., 2007].Mycotoxins before harvest, during harvest and post harvest are produced in different weather condition. When crops have been produced in temperate, Semi-temperate and tropical



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climate or the harvest occurred in high humidity and rainfall conditions, there is a possibility of mycotoxins contamination [Shier W.T et al., 1998]. Limited numbers of mycotoxins such as aflatoxins, zearalenone, fumonisines and ochratoxins in import and export were considered and must be measured. But in some countries these toxins are not measured for animal feeds [Miller R., 1995, Richard J.L., 2007]. Meanwhile permitted levels of mycotoxins present in grain crops and related products (wheat) is still controversial. For this reason, the European Commission and the Human Rights Commission in United Nations strictly have emphasized to prevent or reduce pollution of Fusarium toxins in grains and grain products [Miller R., 1995, Richard J.L., 2007]. Yazdanpanah and colleagues tested a few samples just in Tehran but the present study was covered whole country (Iran) and the low amount of zearalenone in Yazdanpanah et al. must be due to low contamination in that years. The frequency of the samples studied in this research in terms of places shows that the Northern region play an important role in the production of wheat in Iran, Rezapour and colleagues (2012) were detected similar result that matched to our study .

Although the frequencies amount of zearalenone concentration distribution have been considered have significant correlation together but countercurrent correlation. But it should be noted that the highest frequency of zearalenone toxin concentrations in the range of 16-21ppb in all the provinces. Also we can say that Ardebil was not success to reduce the amount of contamination since last year there was same concentration of fusarium contamination. It is true that the contaminations are not very high and life threatening, but all provinces have potential to become more plotted by mycotoxins during transportation and storage. Rashedi and et al (2010) were evaluated zearalenone in Chaharmahal and Bakhtiari rice and wheat samples; they have shown that only 5.7% of 15 samples were contaminated that this finding was similar to Yazdanpanah et al. in our study and Yazdanpanah et al, and Rashedi and et al it is obvious that percentage of zearalenone is not significant for people health [Miller R., 1995, Richard J.L., 2007]. Finally we can see that according to the collected samples in Iran, north of Iran, south and west respectively have the Based on the results of samples collected and the product of the milling process, there is a significant relationship, between pollution below and limited levels (Figure 2). Regarding the results of this research can be said because the main producers of zearalenone is a genus Fusarium. Zearalenone remains at intervals after planting and cultivation; long-term viability of zearalenone residual on grain can cause contamination of farm and food products in years ahead. The pollution rate varies according to geographical regions. But during the process of turning wheat into flour contamination levels of toxins such as zearalenone may be some flour decreased or sometimes increased.

Ahv (Ahvaz Khuzestan), Ard (Ardabil city) Alb (Ali Abad province), Fam (province), Ger (Germi Ardebil province), Gon (Gonbad of Golestan province), Ijr (Ayjrud of Zanjan), Kho (Khodabandeh, Zanjan), Kor (Kordkoy Golestan), KeS (Kermanshah Province), Mog (plain Mog Ardabil province) Nek (Neka, Mazandaran Province), Raz (Razan, Hamedan province), Sar (Sary State Mazandaran).

CONCLUSION AND RECOMMENDATION

Although in this study, no contamination has been detected in the sample that exceeds the standard but according to the high consumption of the food in Iran, further investigation is recommended. This study, one of the first widely assessments of zearalenone flour in the country showed that there is no health risk for consumers in the level of zearalenone.

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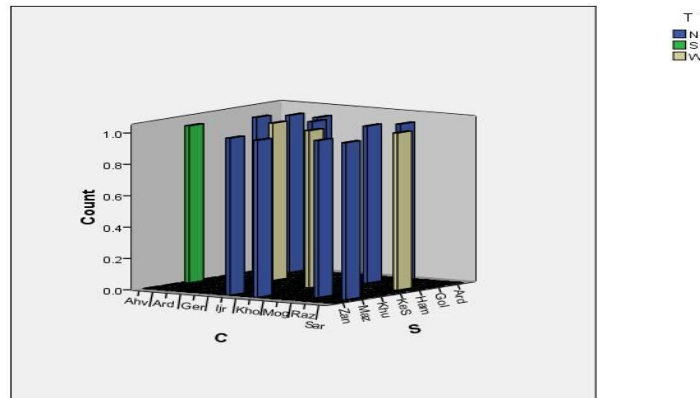


Figure.1: Diagram of sampled cities distribution in the regional provinces of Iran .

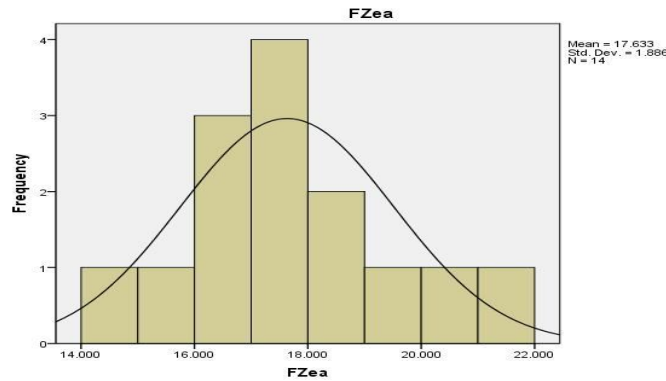


Figure.2.: Frequency graph of the mean FZea toxins, obtained from samples of flour.





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Table 1: Frequency of the taken samples based on cites distribution.

		City			
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Ahvaz	1	7.1	7.1	7.1
	Aliabad	1	7.1	7.1	14.3
	Ardebil	1	7.1	7.1	21.4
	Famenin	1	7.1	7.1	28.6
	Germy	1	7.1	7.1	35.7
	Gonbad	1	7.1	7.1	42.9
	Ijrud	1	7.1	7.1	50.0
	Kermansh	1	7.1	7.1	57.1
	Khodaban	1	7.1	7.1	64.3
	kalaleh	1	7.1	7.1	71.4
	Moghan	1	7.1	7.1	78.6
	Neka	1	7.1	7.1	85.7
	Razan	1	7.1	7.1	92.9
Sari	1	7.1	7.1	100.0	
Total		14	100.0	100.0	

Table. 2: Skewness and Kurtosis-test to check numeric data samples to determine the natural frequency .

Statistics												
	Mean	Std. Error of Mean	Median	Std. Deviation	Variance	Skewness	Std. Error of Skewness	Kurtosis	Std. Error of Kurtosis	Range	Minimum	Maximum
FZea	17.63286	.504059	17.57000	1.886015	3.557	.539	.597	.102	1.154	6.710	14.920	21.630

