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Dear Authors,

It is with much joy and anticipation that we celebrate the launch of special issue VI(S), Volume 4 of Journal of Experimental Biology and Agricultural Sciences (JEBAS). On behalf of the JEBAS Editorial Team, I would like to extend a very warm welcome to the readership of JEBAS. I take this opportunity to thank our authors, editors and anonymous reviewers, all of whom have volunteered to contribute to the success of the journal. I am also grateful to the staff at Horizon Publisher India [HPI] for making JEBAS a reality.

JEBAS is dedicated to the rapid dissemination of high quality research papers on how advances in Biotechnology, Agricultural sciences along with computational algorithm can help us meet the challenges of the 21st century, and to capitalize on the promises ahead. We welcome contributions that can demonstrate near-term practical usefulness, particularly contributions that take a multidisciplinary / convergent approach because many real world problems are complex in nature. JEBAS provides an ideal forum for exchange of information on all of the above topics and more, in various formats: full length and letter length research papers, survey papers, work-in-progress reports on promising developments, case studies and best practice articles written by industry experts.

Finally, we wish to encourage more contributions from the scientific community and industry practitioners to ensure a continued success of the journal. Authors, reviewers and guest editors are always welcome. We also welcome comments and suggestions that could improve the quality of the journal.

Thank you. We hope you will find JEBAS informative.

Dr. Kamal K Chaudhary
Managing Editor – JEBAS
December 2016
Genotypic differences in forage quantity and quality of canopy strata in napiergrass (Pennisetum purpureum Schumach)
doi: http://dx.doi.org/10.18006/2016.4(VIS).688.697

Cloning and expression pattern analysis of MmPOD12 gene in mulberry under abiotic stresses
doi: http://dx.doi.org/10.18006/2016.4(VIS).698.705

Effects of microperforated polypropylene film packaging on mangosteen fruits quality at low temperature storage
doi: http://dx.doi.org/10.18006/2016.4(VIS).706.713

Analysis of phenolic compounds for determination of cambium differentiation and tracheal elements in olive graft combinations
doi: http://dx.doi.org/10.18006/2016.4(VIS).714.720

Effect of different irrigation regimes and zeolite application on yield and quality of silage corn hybrids
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Effect of mulching on soil nutrient loss reduction, case study of western lands Khuzestan province, Iran
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IPM approach for the management of wilt disease caused by Fusarium oxysporum f. sp. Lycopersici on tomato (Lycopersicon esculentum)
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Prevalence of Salmonella spp. in water sources of Sistan: A descriptive cross-sectional study
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Extensive synovial chondromatosis of shoulder: a case study from Shiraz, Iran
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GENOTYPIC DIFFERENCES IN FORAGE QUANTITY AND QUALITY OF CANOPY STRATA IN NAPIERGRASS (*Pennisetum purpureum* SCHUMACH)

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**KEYWORDS**

Canopy structure
Herbage quality
Genotype
*Pennisetum purpureum*

**ABSTRACT**

To assess grazing suitability of napiergrass genotypes across real dwarf Taiwan 7734 (7734), semi-dwarf (DL) and normal-tall Merkeron (ME), yield and quality attributes were determined in canopy strata. Plant densities of 7734, DL, and ME were 4, 2, and 1 plants m⁻², respectively, and relative light intensity (RLI) and dry weight of plant fractions were obtained by stratified clipping at the first and second cuttings in early September and late November, respectively. Results of this study revealed that plant height was in the order of ME (199 cm), followed by DL (128 cm) and 7734 (88 cm) at the first cutting, and 7734 tended to have higher tiller density, dry matter yield, and leaf area index than DL and ME at both cuttings. Canopy RLI in 7734 tended to decrease higher with strata than in DL and ME, which was corresponded with the lowest K in 7734, followed by DL and ME at both cuttings. Genotype 7734 had the highest digestibility and crude protein concentration, and lowest structural carbohydrate concentrations across genotypes, which would be favorable to grazing use by breeding beef cows.

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1 Introduction

Napiergrass (Pennisetum purpureum) is used extensively in Japan as a forage crop for cattle, has a quick growth and produces a high amount of dry matter (Vicente-Chandler et al., 1974). The grass is tolerant to an extensive range of soil conditions, is drought tolerant, and exhibits a high efficiency of photosynthesis and excellent water use efficiency (Anderson et al., 2008). High digestibility of feedstock helps enzymatic saccharification with cellulose (Kai et al., 2010; Knoll et al., 2012; Na et al., 2015).

Napiergrass has a range of phenotypic variation from real-dwarf, semi-dwarf, to normal-tall genotypes. Dwarf type napiergrass (semi-dwarf [dwarf late-heading type; DL]) originated from Florida, in the United States (Sollenberger et al., 1988), was then brought to the Dairy Promotion Organization (DPO) in Thailand, and was finally introduced to Japan in 1996 (Ishii et al., 1998). Although the DL napiergrass has been adopted in tropical and subtropical countries, it has been recently introduced and examined for growth attributes and adaptability to be more suitable for grazing use than normal variety of napiergrass in temperate Japan (Mukhtar et al., 2003; Ishii et al., 2005). In vitro dry matter digestibility (IVDMD), crude protein concentration, and overwintering ability of DL napiergrass were superior to the normal napiergrass genotype in various tropical and sub-tropical areas in the world (Tudsrí et al., 2002). It is important to examine growth attributes along with forage quality of dwarf types in comparison with normal-tall Merkeron (ME) in the region.

Dry matter yield and forage quality suitable for biomass use are expected to be variable, dependent on variations in growth attributes among genotypes of napiergrass, as affected by climate and soil factors at the observed site and dependent on the growth stage of herbage (Woodard & Prine, 1991; Ishii et al., 1998). High leaf expansion, vigorous tillering, and rapid dry matter production in tall canopy are categorized as important factors to attain high production of napiergrass (Ferraris et al., 1986; Matsuura et al., 1991; Wadi et al., 2004).

Little information has been accumulated for variations of growth and forage quality across canopy strata in a range of napiergrass genotypes (Khairani et al., 2013), which is closely related with the solar radiation interception and the efficiency in converting solar radiation to the canopy plant growth (Stejskalová et al., 2013). The present study was conducted to determine yield and quality attributes across several canopy strata for 7734 and DL compared with normal ME napiergrass for estimating carrying capacity of the napiergrass pastures by grazing system.

2 Materials and Methods

2.1 Plot design, Transplantation and Sward Management

The field experiment was conducted at Miyazaki, Japan (E131°25', N31°54') in 2015, using a randomized complete block design (RCBD) with 3 replications. Three genotypes of real-dwarf (Taiwan line 7734), semi-dwarf (DL), and normal-tall genotype, ME napiergrass were selected. Plot size was fixed at 6 m² (2 m × 3 m) for both DL and 7734, while for ME, plot size was fixed at 12 m² (3 m × 4 m), which had 60, 105, and 12 plants per plot for DL, 7734, and ME, respectively. Density and spacing of plants were 1 plant m⁻² with 1 m × 1 m spacing for ME, 2 plants m⁻² with 1 m inter-row with 0.5 m intra-row spacing for DL, and 4 plants m⁻² with 0.5 m of both inter-row and intra-row spacing for 7734. The previous crops were removed, and cow manure at 3 kg m⁻² and slaked lime at 150 g m⁻² were added on April 22nd, 2015. Rooted tillers of 7734, DL, and ME were transplanted at the density of 4, 2, and 1 plants m⁻², respectively, on May 26th-27th, 2015. Chemical compound fertilizer at 5 g each of N, P₂O₅, and K₂O m⁻² was supplied twice before the first cutting and just after the first cutting on September 4th for an annual total of 15 g each of N, P₂O₅, and K₂O m⁻². Weeds were removed by hand as required.

2.2 Sampling methods and growth characters to be determined

Growth attributes including plant height, plant length, tiller density, and leaf area were measured for 2 plants per plot on June 15th, July 24th, September 4th, October 15th, and November 28th, 2015. Samples were randomly selected and plants were cut at 10 cm above the ground as reported by Ishii et al. (2005) to measure fresh weight (FW). From these, subsamples approximately 300–400 g FW were separated into leaf blade (LB), stem inclusive of leaf sheath (ST), and dead part (D) and then oven-dried at 70°C using the ventilation oven (model DKM 600, Yamato Scientific Co. Ltd, Tokyo, Japan) for 3 days (72 hours) to determine the percentage of dry matter (DM) in each plant fraction. Plants were measured for relative light intensity (RLI) in every 30 cm strata from the top of the canopy to the ground and harvested by the stratified clipping method of 30 cm strata to measure FW and percentage of DM on September 4 for all genotypes at the first cutting and on November 28 for ME at the second cutting. The width of strata decreased to 20 cm for DL and 7734 due to lower plant height on November 28. The bottom strata were harvested at 10 cm above the ground.

2.3 Chemical analysis of herbage

The ground samples for 3 genotypes of napiergrass were passed through a 1 mm screen in herbage for LB and ST. They were then analyzed for in vitro dry matter digestibility (IVDMD), crude protein (CP), and structural carbohydrates such as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) by detergent methods (Van Soest, 1994). IVDMD was measured in the case of duplication by pepsin-cellulose digestion method (Goto & Minson, 1977) using the in vitro incubator (Model: ANKOM DAISY II, ANKOM Technology, New York, USA).
2.4 Statistical analysis

Analysis of variance was performed using Excel Statistics (OMC Co. Ltd., Saitama, Japan). Differences in mean values were assessed at the 5% probability level using the least significant difference (LSD) method.

3 Results and Discussion

3.1 Climatic Conditions

Monthly mean temperature and precipitation in 2015 are shown in Figure 1, compared with those in the normal year (NY) averaged from 1981 to 2010 data determined at Miyazaki Meteorological Observatory in Japan Meteorological Agency (Japan Meteorological Agency). The mean temperatures in June and July 2015, respectively, were lower at 21.8°C and 25.7°C than those in the NY at 23.1°C and 27.3°C. Monthly precipitation in June and July, 2015 was 840 and 573 mm, respectively, which was higher than those in the NY at 429 and 309 mm month$^{-1}$. Monthly mean temperature and precipitation in August and September, 2015 were similar to those in the NY. Therefore, the climatic conditions were lower temperature and higher precipitation in June and July than in the NY. In addition, a drought condition appeared at 19 mm precipitation in October, 2015 compared with 182 mm in the NY, which suppressed plant growth severely.

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Figure 1 Changes in climate conditions in 2015 compared with the normal year (NY), averaged for 1980-2010.

For the change in plant height for the 3 genotypes in 2015, Figure 2 shows the mean values ± standard deviation (n=3). Cutting. Symbols with different letters denote significant difference at the 5% level by LSD test. ns: $P > 0.05$. 

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Genotypic differences in forage quantity and quality of canopy strata in napiergrass (*Pennisetum purpureum* Schumach).
3.2 Changes in growth attributes

Changes in plant height per month across the 3 genotypes 7734, DL, and ME are shown in Figure 2. Plant height was generally larger in the normal ME than the semi-dwarf and the dwarf genotypes (Mukhtar et al., 2003; Khairani et al., 2013), showing the highest in ME (199 cm), followed by DL (128 cm), and 7734 (88 cm) at the first cutting on September 4th, while the order between the semi-dwarf and dwarf genotypes was reversed (105 cm for 7734 and 79 cm for DL) at the second cutting on November 28th. In the regrowth period after the first cutting, probably due to higher sensitivity to short day length in 7734, the day length might trigger the ear initiation in the dwarf 7734, which should release the suppression of internode elongation, resulting in a higher plant height in 7734 than in DL at the second cutting.

Changes in tiller density per month across the 3 genotypes are shown in Figure 3. Tiller density in 7734 was constantly higher across growing seasons than the other 2 genotypes, showing the maximum at 175 m$^{-2}$ in 7734, followed by DL and ME, which was positively correlated with the difference in plant density among the 3 genotypes. Decrease in tiller density from October to November in 7734 may be caused by self-shinning of tillers (Matsuda et al., 1991) from the maximum tiller density in mid-October, while the other genotypes maintained tiller density from October to November.

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Figure 3 Changes in tiller density for the 3 genotypes in 2015. The symbol shows mean values ± standard deviation (n=3). ↓: Cutting. Symbols with different letters denote significant difference at the 5% level by LSD test.

Figure 4 Changes in dry matter yield of the 3 genotypes in 2015. The symbol shows mean values ± standard deviation (n=3). ↓: Cutting. Symbols with different letters denote significant difference at the 5% level by LSD test. ns: P > 0.05.
Changes in DM yield in the 3 genotypes across the growing season are shown in Figure 4. DM yield was almost constantly higher in 7734, followed by ME and DL, except for when ME had the lowest weight in October. In the first cutting on September 4th, 7734 had the highest DM yield at 830 g m⁻², followed by ME at 640 g m⁻², and DL at 590 g m⁻². In the second cutting, DM yield was not significantly different among cultivars. Therefore, annual total DM yield was significantly higher in 7734 at 1223 g m⁻², followed by ME at 906 g m⁻², and DL at 793 g m⁻². The slow recovery on October 15th from the first cutting may be adversely affected by the lowest monthly precipitation (19 mm) in October, which was abnormally lower than the NY monthly precipitation (182 mm). On the other hand, management of N fertilizer is the most effective tool in enhancing and manipulating both herbage yield and quality in normal genotypes (Broyles & Fribourg, 1958; Boonman, 1993) as well as in dwarf genotypes (Muhammad et al., 1988; Wadi et al., 2004). Utamy et al. (2011) reported that DM yield in DL was so variable across the observed sites in southern Kyushu, ranging from 70–1360 and 20–1580 g m⁻² year⁻¹ in 2007 and 2008, respectively. It is clear that significantly positive correlation was obtained from the study between DM yield and nitrogen fertilizer supply. In the present study, annual fertilizer supply was limited to 15 g m⁻², which might be suboptimal to normal ME and semi-dwarf DL.

Changes in dry matter partitioning of plant fractions (LB, ST, and D) in the 3 genotypes are shown from June 15th to November 28th in Figure 5. Percentage of LB was higher in the second than in the first cutting for all genotypes, while the ST percentage and D percentage were higher in the first than in the second cutting. Percentage of LB was higher in DL than in 7734 and ME at both cuttings, except for June 15th when the highest LB (100%) in both DL and ME occurred, showing a simple index for crude protein concentration of herbage. LB percentage tended to increase from the first to the second cuttings across the 3 genotypes, corresponding with the previous study for DL (Hasyim et al., 2014), showing that the ratio of leaf blade to stem (LB/ST) was lower at the first cutting in the year of establishment than at the other 2 cuttings and tended to decrease with increasing DEM application across the seasons. In the first defoliation of DL napiergrass, when the plant height reached 111–132 cm, DM yield and LB percentage were recorded at 226–717 g DM m⁻² and 61-87%, based on early pasture management practices for prompt weeding and fertilization (Ishii et al., 2013), which was comparable to the present DL in the first cutting.

3.3 Canopy structure

Changes in the relative light intensity (RLI) of the canopy and canopy architecture were observed in the first and second cuttings on September 4th and November 28th, respectively, across the 3 genotypes (Figure 6). Canopy RLI decreased with strata, and the decreased percentage tended to be more severe in 7734 than in DL and ME. Stratified clipping was conducted at every 30 cm strata for all genotypes in September and for ME in November, when the clipping at 20-cm interval was applied to 7734 and DL due to lower plant height. The LB biomass yield gradually increased from the upper to the bottom strata for each genotype in the first and second cutting, except for the lowest strata, which had lower yield. The ST biomass yield peaked in the lowest strata for all genotypes. Even though RLI tended to decrease slowly in the second cutting on November 28th, the amount of leafages was lower in the second cutting on November 28th than in the first cutting on September 4th.
Table 1 Crude protein concentrations (mg g\(^{-1}\) DM) of plant fractions in napiergrass genotypes.

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Strata: 2 (Upper) to 7 (Bottom). LB, leaf blade; ST, stem inclusive of leaf sheath. Figures with different letters denote significant difference at the 5\% level by LSD test.

Figure 6 Changes in relative light intensity (RLI) in the canopy and canopy architecture at the first cutting on September 4 (I) and at the second cutting on November 28 (II). The symbol shows mean values (n=3).
The canopy extinction coefficient (K) was the lowest in 7734 at 0.36 and 0.61 for the first and second cuttings, respectively, followed by DL at 0.56 and 1.06 and ME at 0.60 and 1.15 for the first and second cuttings, respectively. Therefore, canopy K increased from September to November in all genotypes, reflected by a lower leafage amount in November (Nagasuga et al., 2002), while K was the lowest in 7734, followed by DL and ME commonly at both cuttings due to the steeper leaf angle in 7734 than in the other genotypes.

It might be possible that through seasonal variations in LAI and K, the canopy of napiergrass can maintain higher efficiency, even when solar radiation is intercepted for a longer time during its growth. Zhang et al. (2014) showed that K is an important factor that affects carbon fixation of the ecosystem, as well as water and energy transmission. A low K indicates that a lot of radiation can reach the bottom of canopy strata, while a high K indicates that only a little radiation can penetrate to the bottom of the canopy. Zhang et al., (2014) reported that cropland had the highest K (0.62), followed by broadleaf forest (0.59), shrubland (0.56), and grassland (0.50) across the several ecosystems. In the present study, the average K for all genotypes at both cuttings was 0.72, while 7734 had the lowest K at 0.36 at the first cutting, which was superior to that in the grassland (Zhang et al., 2014). Annual mean K values were higher in the normal than in the dwarf genotypes among different planting densities (Mukhtar et al., 2003), which was consistent with the present study.

3.4 Forage Quality in Canopy

Changes in crude protein (CP) concentration of plant fractions for LB and ST in every strata of the 3 genotypes were determined for the first and second cuttings on September 4th and November 28th, respectively (Table 1). CP concentration of both LB and ST were higher in the second than in the first cutting in all 3 genotypes, except for lower CP concentration in ST for ME. In general, CP concentration was the highest in 7734, ranging from 82 to 145 mg g⁻¹ DM for LB and 91 to 102 mg g⁻¹ DM for ST in the first cutting and was increased to 114 to 206 and 91 to 181 mg g⁻¹ DM for LB and ST, respectively, in the second cutting, followed by DL and ME. CP concentration in ME ranged from 90–183 and 60–180 mg g⁻¹ DM in LB and ST, respectively, and LB tended to have higher CP concentration than ST (Fukagawa et al., 2000). The dwarf genotypes of napiergrass tended to have higher CP concentration than the normal genotypes (Sollenberger et al., 1988; Muiniga et al., 1993; Silva et al., 1994; Chaparro & Sollenberger, 1997; Tudsr et al., 2002), which is consistent with the present study. CP concentration was higher in the upper than in the bottom strata for every genotype, which is closely related with animal performance when napiergrass is used for grazing (Silva et al., 1994).

Changes in in vitro dry matter digestibility (IVDMD) of plant fractions for LB and ST in every strata of the 3 genotypes were determined at the first and second cutting in September and November, 2015, respectively (Figure 7). IVDMDs of both LB and ST were higher in dwarf 7734 and semi-dwarf DL than in the normal ME in the first and second cuttings. In 7734, IVDMD was higher for LB in the first than in the second cutting, while ST had a higher IVDMD in the second than in the first cutting. In DL, IVDMD for LB was lower in the first than in the second cutting, while ST had higher IVDMD in the first than in the second cutting. In ME, IVDMD for LB was higher in the second than in the first cutting. Therefore, IVDMD was the highest in 7734, followed by DL and ME, tended to be higher in LB than in ST, and was higher in the upper than in the bottom strata for each genotype.
Table 2 Structural carbohydrate concentration (mg g⁻¹ DM) of plant fractions in NDF (A), ADF (B) and ADL (C) for the 3 napiergrass genotypes.

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Strata: 2 (Upper) to 7 (Bottom), LB, leaf blade; ST, stem inclusive of leaf sheath. Figures with different letters denote significant difference at the 5% level by LSD test.

It is reported that IVDMD in ME was variable from 567–772 and 619–786 mg g⁻¹ DM in LB and ST, respectively, indicating that ST tended to have higher digestibility than LB (Fukagawa et al., 2000).

In normal ME, digestibility tends to be higher in ST than in LB at the juvenile stage, while this tendency is reversed in the mature stage, since the decreasing rate in digestibility of ST during maturing was larger than that of LB. As for semi-dwarf DL, IVDMDs in LB ranged from 570–712 and 560–681 mg g⁻¹ DM in 2007 and 2008, respectively, while those in ST were higher than those in LB, and ranged from 619–747 and 637–765 mg g⁻¹ DM in 2007 and 2008, respectively (Utamy et al., 2011).

Changes in structural carbohydrates concentration were determined in every strata at the first and second cutting in September and November, 2015, respectively (Table 2). Structural carbohydrate concentrations in NDF, ADF, and ADL (Lignin) were the lowest in 7734, followed by DL and ME in the first cutting, while in the second cutting, NDF, ADF, and ADL concentrations followed the same order as those in the first cutting, except for ME, which had a lower concentration than DL. NDF and ADF concentrations were
lower in LB than in ST for DL and ME, and these concentrations were lower in the upper than in the bottom strata of each plant fraction for all 3 genotypes for both the first and second cutting. Aroeira et al. (1999) reported that, for normal napiergrass, NDF concentration ranged from 688 to 752 ± 2.2 mg g⁻¹ DM, and ADF concentration ranged from 383 to 439 ± 2.9 mg g⁻¹ DM, with the highest values generally found in the summer. Similar fiber contents were reported for the dwarf genotype (Silva et al., 1994).

Conclusions

Under the highest plant density (4 plants m⁻²), the dwarf 7734 achieved the comparative yielding ability to DL and ME, due to the steeper leafage of the canopy. Forage quality tended to be the highest in 7734, followed by DL and ME in terms of IVDMD, and concentrations of CP and structural carbohydrates. The results suggest that the 7734 genotype is the most efficient type of napiergrass for both forage quality and quantity.

Acknowledgements

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

References


CLONING AND EXPRESSION PATTERN ANALYSIS OF MmPOD12 GENE IN MULBERRY UNDER ABIOTIC STRESSES

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Mulberry
MmPOD12 gene
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Abiotic stress

ABSTRACT

A full-length cDNA denominated as MmPOD12 for peroxidase in mulberry (Morus alba), an enzyme involved in the respiration, photosynthesis, and the oxidation of auxin, was cloned from ‘Yu71-1’ a variety of mulberry using a rapid amplification of terminal (RACE) approach. The full cDNA of MmPOD12 has 1482 base pairs (bp) in length with an open reading frame (ORF) 1050 bp encoding a protein of 349 amino acids residues with a predicted molecular weight of 53.92 kDa and an isoelectric point of 9.35. Sequence analysis revealed that MmPOD12 shares homology with Morus notabilis (M. notabilis C.K. Schm) and has closely related to green plum, strawberry and pear. The expression patterns of MmPOD12 treated with drought, salt and hormones stresses were examined using real-time quantitative PCR (RT-qPCR). These experiments caused significant up-regulation of the expression of MmPOD12 under drought and salt stress. The highest expression level of MmPOD12 appeared at 2d for salt stress, and 7d for drought stress, while a significant fluctuation of MmPOD12 expression was detected after ABA and SA stresses. These findings provide a basis for future functional analyses of MmPOD12 gene in Mulberry.

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1 Introduction

Peroxidase (POD) widely exists in biological world and it plays an extremely important physiological role in plants as it presents from the first hours of a plant’s life until its last moments. POD is not the single-minded enzyme of hydrogen carrier except for H₂O₂ but it can also catalyze the oxidation reaction of Phenolic, Cytochrome C, Vitamin C, Nitrite, Colorless dye, Indole, Amine and Inorganic ions (especially Iodide ion) with H₂O₂. Besides, due to POD can catalyze the decomposition reaction of IAA, a kind of hormone that can promote plant cell elongation, it also played a role in plant tissue differentiation, seed germination and fruit maturity (Passardi et al., 2005).

In addition, POD is very sensitive to the variety of adverse environmental conditions. POD isoenzyme will be produced quickly when plants are infected by bacteria, and rapid induction of POD isoenzyme was related to the denovo synthesis according to the experiments about protein synthesis inhibitors (Hiraga et al., 2001). The level of POD activity was higher when plants were treated with low concentration of ethylene or attacked by pathogens (Tognolli et al., 2002). There are many studies which demonstrated that POD can eliminate the effect of injury caused by H₂O₂ during the processes of metabolic in plants (Wu & Yu, 1994; Liang et al., 2003). Further, some studies suggested that POD activity of litchi fruit stored at room temperature was higher than the wet storage. POD have multi functional gene in Ginkgo biloba as it has the potential function on defense aspects, such as it participates in the removing of heavy metal pollution and dealing with the damage (Cheng et al., 2010).

Under some abiotic stress, the activity and expression level of POD were affected. There are some reports which asserted role of POD in overcoming salt stress in plants and the level of POD increased with inducing some stress (Zhu, 2002; Narayanan et al., 2005; Dai et al., 2015). Further, Dai et al. (2015) observed higher POD activities in leaves of Asparagus bean seedling immediately after inducing salt stress by NaCl (150 mmol/L). There’s a study which found POD activity in leaves of Populus Euphratica and it increase with the aggravation of drought stress (Wang et al., 2013). Other papers also reported that drought stress induced the level of POD activity in Crassulaceae plant (Wen et al., 2014) and Lagerstroemia indica seedling (Liu et al., 2015). Studies on role of gene expression on POD activity suggested that under stresses of Absciscic acid (ABA) and Salicylic acid (SA), the level of POD activity change (Fang et al., 2014; Fang et al., 2014; Xu et al., 2015). Study on the effect of exogenous ABA induced stress on the seed germination and cold tolerance of winter rape seedlings, showed that the level of POD activity significant increased and the cold resistance is optimum when ABA concentration is 30 mg/L (Fang et al., 2014).

In this study, cloning of the POD12 gene in Mulberry based system on the expressed sequence tags (ESTs) from mulberry cDNA library was constructed by following the method of Zhao (2008) and Fang et al. (2008), and we named it MmPOD12. The purpose of this study is to got the full-length sequence and analyzed the expression of the gene under different kinds of stresses in mulberry. These results provide a new breeding strategy for improving mulberry resistance.

2 Materials and Methods

2.1 Plant materials

Mulberry (Morus alba) variety ‘Yu71-1’, obtained from the Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang, China, was used as the experimental material. To analysis MmPOD12 gene expression pattern under various stresses, ‘Yu71-1’ grafting seedlings were grown under standard conditions that the temperature is maintained at 25℃ and the photoperiod is 12 h in an incubator, until the winter shoots reached approx. 30 cm in length (50 d).

2.2 RNA isolation and synthesis of the first strand cDNA

Total RNA was isolated from fresh buds (approx. 90 mg) of the grafted Mulberry seedlings using the RNaprep Plant kits (TaKaRa Biotechnology Co. Ltd., Dalian, P. R. China) and following the manufacturer’s protocol, and then stored at -80℃ re-suspended in 0.1% (v/v) diethylpyrocarbonate (DEPC)-treated water. RNA quality was determined with UV spectrophotometer and by 1.0% (w/v) agarose gel electrophoresis.

The first strand cDNA was synthesized from 9μl total RNA (1.0 ng μl⁻¹) from the previous step using the RNase H-Reverse Transcriptase M-MLV kit (TaKaRa Biotechnology Co. Ltd.). Following the manufacturer’s instructions, the reaction conditions are 42℃ for 60min with 4.0μl oligo-dT (100μg μl⁻¹) and adaptor primer in a total volume of 20μl.

2.3 Molecular cloning of the full-length MmPOD12 cDNA

The first strand cDNA was used as the template for PCR in gene cloning. The forward and reverse primers were designed according to the EST with the inference function from the mulberry cDNA library (MmPOD12 Forward primer: 5'-TAGATGCCACCGACACGGT-3'; MmPOD12 Reverse primer: 5'-ACTTGGATTCCTAGCAGAGC-3'). The RT-PCR reactions system were performed in a total volume of 50μl contained 1.0μl first-strand cDNA,41μl ddH₂O,1μl each gene-specific primer,0.5μl dNTPs (10mM),5μl buffer, and 0.5μl Taq DNA polymerase (5U/ml) (TaKaRa Biotechnology Co. Ltd.). The PCR amplification condition: initial denaturation at 94℃ for 5 min firstly; then there are 32 cycles of denaturation at 94℃ for 35 s, annealing at 60℃ for 45s,and elongation at 72℃ for1 min; finally, extension at 72℃ for 10 min.

The RT-PCR products were detected by gel electrophoresis (1% agarose gels) and purified following the Takara Agarose
In 3′ end reverse transcription reaction, the cDNA which was synthesized from 9 µL total RNA by Reverse Transcriptase M-MLV (RNaseH-) at 42°C for 1h with 4 µL 3′ap primer (in a total volume of 20µl), was used as a template. The gene-specific primer is GSP1: 5′-ACAAACACGCACTGCGCAAC-3′, 3′RACE-PCR amplifications condition: denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 45 s, and elongation at 72°C for 60s; with a final extension at 72°C (Tong et al., 2013).

The 5′ end reverse transcription reaction following the instructions of SMART™ RACE cDNA Amplification Kit (CLONTECH Co. Ltd.). The 5′-RACE reaction system as followed: 1µl cDNA template, 17.25µl ddH2O, 1µl 5′ end specific primer (GSP2: 5′-ATGACAGGGTCTTGGGTGGGATAGAG), 1µl common primer, 2µl dNTP, 2.5µl buffer, and 0.25µl rTaq DNA polymerase (5U/mL), in total 25µL; and the reaction condition is that: initial denaturation at 94°C for 5 min; then 5 cycles of denaturation at 94°C for 30 s, annealing at 72°C for 30 s, elongation at 72°C for 2 min; then 5 cycles of denaturation at 94°C for 30 s, annealing at 68°C for 30 s, elongation at 72°C for 2 min; and then followed by 25 cycles of denaturation at 94°C for 30s, annealing at 66°C for 30s, elongation at 72°C for 2 min; and the final extension at 72°C for 7 min. The 3′ and 5′ ends RACE products were all analyzed by Gel electrophoresis.

2.4 Bioinformatics analysis of the MmPOD12 gene

The cloned gene sequence was analyzed for identity and homology using the National Center for Biotechnology Information (NCBI) online Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/). And the ORF of MmPOD12 was using the ORF finder program at NCBI (http://www.ncbi.nlm.nih.gov/orf/orf.cgi) and downloading the homology nucleotide sequences from the database. Then use the DNA Star software to splice various DNA fragments and analyses it.

The MmPOD12 protein structure and functional domains were predicted using the tools of ExPASy (http://prosite.expasy.org/psort.html/) and PSORT the software on line. The molecular mass and the theoretical isoelectric point were predicted using the software DNASTar and ExPASy-ProtParam (http://web.expasy.org/protparam/). Subsequently, using the ClustalX program and DNAMAN software to align and compare the multiple amino acid sequences of POD genes from different species. The on line software SWISS-MODEL (http://swissmodel.expasy.org/) was used to the prediction of MmPOD12 protein tertiary structure. Finally, software MEGA5.1 and clustalx were used to generate the phylogenetic tree of the POD proteins from different species by the neighbor-joining (NJ) method. The bootstrap analysis based on One thousand replicates, and the Protein domains were predicted using SMART (http://smart.embl-heidelberg.de/) package.

2.5 MmPOD12 expression patterns under stresses using qRT-PCR

Mulberry (M. alba) ‘Yu71-1’ grafting seedlings were planted in the same specification pots, and each pot containing only one seedling. The growing conditions were controlled with a 16h photoperiod and 25°C/22°C (day/night, respectively). After approximately 2 months, when the shoots had reached approx. 20 cm in length, the seedlings were subjected to salt (0.3mol/LNaCl, irrigate), drought (PEG-6000, 20%),ABA (0.1mol/L, spray) and SA (0.1mol/L, spray) respectively.

The leaf samples (approx. 1.0g) were collected in 6h, 12h, 1d, 2d, 3d, 4d, 16d after the initiation of salt treatment; in 2d, 4d, 8d, 10d, 16d after the initiation of drought treatment; and in 2h, 4h, 6h, 8h, 10h, 1d, 2d, 3d after the initiation of ABA and SA treatment respectively.

To reveal the putative biological function of the MmPOD12 protein, qRT-PCR method was used to analysis the expression levels of MmPOD12 gene under the four abiotic stresses. The first-strand cDNA was reversely transcribed from total RNA as the directions of the Prime script RT Reagent Kit (TaKaRa Biotechnology Co. Ltd, Dalian, P. R. China), and PCR was carried out following the SYBR Premix Ex Taq Kit(TaKaRa Biotechnology Co. Ltd.) directions. The reaction system was performed in total 20µl volume, containing 10µl SYBR® Premix Ex TaqTM,0.5µl Rox Reference Dye (50x),0.5µl each primer solution, 1.5µl reverse transcription product, and then add RNase-free water. The mulberry Maactin gene (β-actin) (GeneBank access No. DQ785808) was used for internal control to allow for normalization by visual inspection of mRNA level. The Forward primer (β-actin-F: 5′-GACAATGGAACTGGAATGG-3′) and the Reverse primer (β-actin-R: 5′-GACCCCTTCAAATCCAGACA-3′) were used for PCR amplification. The reaction conditions as follows: first, initial denaturation at 95°C for 10min; then, followed by 45 cycles of denaturation at 95°C for 15s, annealing at 58°C for 20s, extension at 72°C for 20s; and then at 95°C for 15s, at 60°C for 1min; finally, end at 95°C for 15minutes.

The result of PCR was analyzed by the Applied Biosystems 7300 System SDS Software and analyzing RT-PCR data by the comparative CT method (Livak & Schmittgen, 2001). Then calculating the average values for MmPOD12 gene expression in the two biological replicates, and the standard errors.
3 Results

3.1 Gene cloning and bioinformatics analysis of MmPOD12

The mulberry cDNA library screening technique and the clone technology combining bioinformatics analytical methods were used to obtain a cDNA of the Mulberry POD gene and the RT-PCR products were analyzed by gel electrophoresis (1% agarose) (Gao et al., 2010) and get a expected size (approximately 500 bp) band was determined according to the EST sequence (Figure. 1A) (Fang et al., 2008). 3' and 5' RACE technology was used to obtain a full-length cDNA of Mulberry POD gene, which contained a 780 bp fragment (3'-RACE product, Figure. 1B) and a 750 bp fragment (3'-RACE product, Figure. 1C), then the full-length cDNA of the mulberry POD gene was obtained. This is the first time cloned POD gene of mulberry, and it was designated the gene as MmPOD12 (Patent number:101510163922.9). Sequence analysis showed that the isolated cDNA from 'Yu71-1' is a small gene with a 1482 bp full-length, which contains a 1050 bp ORF encoding a protein of 349 amino acids, and it was predicted that the molecular weight of the protein is 53.92 kDa and the isoelectric point is 9.35 (Figure. 2).

According to software Batch CD search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), we find that there is a conserved domain contained by MmPOD12 gene structure. This conserved domain can be regard as position-specific score matrices (PSSMs) when identifying the conserved domains in protein sequences by RPS-BLAST.

3.2 Sequence analysis of MmPOD12

The homologous alignment and deduced amino acid sequences of MmPOD12 were analyzed using the software Clustal X2.0 program (http://www.clustal.org/) and Vector NTI Advance 11. The results showed that the length and structure of MmPOD12 homologous sequences are relatively similar with other seven species. Homology analysis revealed that MmPOD12 is highly conserved among mulberry different species, but there are three different amino acids in ‘Yu71-1’ and Morus notabilis, this mean there are particularities in different species.

According to the MmPOD 12 amino acid sequence structuring the evolutionary tree by MEGA5.1 Software. The results was visualized in Figure. 3 that the length and structure of the16 species amino acid sequences are very conservative. There is relatively close homology between ‘Yu71-1’ and Morus notabilis, but the homology is far between ‘Yu71-1’ and Eucalyptus grandis, Grape, Populus euphratica, Populus trichocarpa and Litchi.

3.3 Stress-induced MmPOD12 gene expression

To further analysis whether the level of MmPOD12 gene expression is induced by various abiotic stresses, it was monitored that the MmPOD12 mRNA levels under abiotic stresses by qRT-PCR (Figure.4) illustrates that, the expression level of MmPOD12 gene fluctuates obviously under salt stress, the overall trend is decreasing at the initial of salt treatment, followed there is a sharpest rise and up to a maximum, and then decrease slowly (Figure.4A).
AGCGAAAGCTTTGTATATAATAAATGATTTACCCACCATAGTGCATAAAAAGTGCTGCTGATGAAGTCTCTTGGTCTAATATCACTGCTTTCGACGCGATGATGCTCTTGGTCTAATATCACTGCTCTTGCAGCGCATGATTCTGTTTTCCTGCTTCGTGGTGGTGACTATGCATCACGATCACCTGAA

AGCGAAAGCTTTGTATATAATAAATGATTTACCCACCATAGTGCATAAAAAGTGCTGCTGATGAAGTCTCTTGGTCTAATATCACTGCTCTTGCAGCGCATGATTCTGTTTTCCTGCTTCGTGGTGGTGACTATGCATCACGATCACCTGAA

ATG indicates the start codon; TAA indicates the stop codon.

After the drought stress, the expression level of *MmPOD12* gene change little at the initial days, it begin to increase in 8d and continued to grow (Figure 4B). The expression level of *MmPOD12* underABA and SA stresses showed a significant wave as it increase firstly and then decrease (Figure 4C and Figure 4D). According to the qPCR analysis, we initially speculated that *MmPOD12* is related to mulberry resistance, especially in salt and drought stresses.

Figure 2 The nucleotide and deduced amino acids sequence of mulberry *MmPOD12*.

Figure 3 The phylogenetic tree based on amino acid sequence of *MmPOD12* and other homologues sequences.
4 Discussions

In this study, a full-length cDNA sequence of POD from mulberry variety ‘Yu71-1’ was obtained for the first time by cloning technology. It was analyzed the gene’s sequence, expression pattern and predicted the protein coded by it and explored the expression level of MmPOD12 under various abiotic stresses. In this study, it was reported that MmPOD12 can compared with the POD in other species with a higher homology, an identical conserved regions and a similar tertiary structure. When mulberry plants were in adverse environments such as salt and drought, the expression level of MmPOD12 will adjust to response to environmental stresses.

The adjustment mechanism of MmPOD12 was understood preliminarily. The results of qRT-PCR analysis shows that the expression level of MmPOD12 mRNA significantly increased under salt stress, and the expression level up to the highest in 6 h after treatment. This indicates that the adversity like salt affect MmPOD12 gene expression and over expression. This present result was consistent with the previous reports that the POD genes played an important role during the plants’ physiological mechanisms adapting to salinity change (Du et al., 2011).

Under drought stress, the transcriptional level of MmPOD12 mRNA will increase gradually with the extension of time and there is a sharp increase peak at the later period. This indicates that, under mild and moderate drought treatment, MmPOD12 activity was modest overall increase as the plants against the damage caused by drought stress through other path mainly; and when mulberry plants under severe drought environment, MmPOD12 activity increased significantly as it participating in resisting the drought stress. This is consistent with other species with strong drought resistance (Peng et al., 2005).

Under ABA and SA stresses, the expression levels of MmPOD12 increased, and the effect of SA more obvious than ABA. This suggests that spraying exogenous ABA can improve POD activity and this is consistent with the study result of Fang et al. (2014).

To summarize, a full length cDNA of MmPOD12 was isolated from mulberry variety ‘Yu71-1’ and characterized in this paper. Multiple alignments and bioinformatics analysis results showed that the deduced MmPOD12 had high similarity to other plant PODs. Expression profiles of MmPOD12 under different treatments suggest that MmPOD12 was a stress-responsive gene, especially to salt and drought. The cloning,
characterization and expression analysis of \textit{MmPOD12} will be helpful to understand more about its role in the resistance to stresses for plant, which provide the basis for improving the ability to anti-stress by genetic manipulation in the near future.

\section*{Acknowledgments}

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\section*{Conflict of interest}

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

\section*{References}


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EFFECTS OF MICROPERFORATED POLYPROPYLENE FILM PACKAGING ON MANGOSTEEN FRUITS QUALITY AT LOW TEMPERATURE STORAGE

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KEYWORDS

Polypolyene
Lifespan
Storage
Mangosteen
Quality

ABSTRACT

The study was conducted to explore the effects of micro-perforated polypropylene (PP) film bag with different numbers of perforations (holes) on the quality of mangosteen fruit stored for 25 days at 13°C. Further, study was aimed to identify a best suitable method for packaging mangosteen which can improve the storage time along with maintaining the fruit quality. In this study fruits were packed in expanded PP perforated with different numbers of holes i.e. 10, 20, 30 and 40 per bag, and the results were compared with the commercially available MAP-Lifespan bag and non-bagged treatment. The experiment was carried out in completely randomized design with 4 replications and 12 fruits per replication. After 10, 20 and 25 days of storage, gas composition of package and physicochemical qualities of the fruit were determined. The results of study revealed that fruits stored in polypropylene bags with 30 pores (PP30) could maintained the postharvest quality of mangosteen up to 25 days at 13°C and reported more effective than the MAP Lifespan bag. These results can recommended that PP30 can use for commercial purpose.

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1 Introduction

Mangosteen (Garcinia mangostana L.) is a tropical fruit with a high economic value and also known as a queen of tropical fruits (Wieble et al., 1992; Deewatthanawong et al., 2003). Although fruit has great economic value but short shelf life has a biggest obstacle in its wide scale consumption and transportation. Normally it has 3-7 days shelf life at room temperature and it can extend up to 7-14 days at lower temperature such as 13°C or below this (Ketsa & Koolpluksee, 1993). Common disorders which occurred during the storage of this fruit are browning of the pericarp (peel), weight loss, shrinkage of the calyx and pericarp at the stem end and chilling injury (Deewatthanawong et al., 2003). In addition, reduction in the internal fruit quality was also reported. These changes resulted in reduced marketability and loss of economic value of the fruit (Deewatthanawong et al., 2003). Use of modified atmosphere packaging (MAP) in storage can provide significant advantages. This MAP method reduced the rate of respiration; make delayed in ripening and aging process and also reduced the water loss from the fruit which finally increase the storage life of fruit. According to Manurakchinakorn et al., (2008) mangosteen fruit can retained acceptable eating quality for 28 days if these were harvested at ripeness Stage 1 (light greenish-yellow skin and 5-50% scattered pink spots) and packed in LDPE with 6% O₂: 15% CO₂ atmosphere at 13°C . Similarly, Pakkasarn et al. (2003) reported that the storage of mangosteen fruits at 13°C under an atmosphere of 10% CO₂ caused delay in peel color changes and firmness which finally caused reduction in weight loss and rate of respiration and maintained fruit quality for 28 days. No information is available on the use of specialized packaging materials in Vietnam for maintaining the quality of mangosteen fruit during the storage or transport to distant markets.

Although bags used for packaging are capable in delivering a modified atmosphere (MA) and are effective in controlling in-package gas composition, respiratory rate and physicochemical changes e.g. commercially available MAP Lifespan bag but the cost of such bags are very high and it is difficult to obtain these bags for commercial use. This study aimed to compare the use of polypropylene (PP) film bags with different numbers of micropores (holes) with the MAP Lifespan bag on the composition of the atmosphere and physiological changes of Vietnamese mangosteen during low temperature storage.

2 Materials and Methods

2.1 Fruits and packaging materials

2.1.1 Fruit characteristics

Mangosteen fruits were harvested at Stage 2 maturity with light greenish-yellow skin and 51-100% scattered pink spots as suggested by Tongdee & Suwanagul (1989) (Figure 1), from a mangosteen orchard situated at Cho Lach district, Ben Tre Province, Mekong Delta, Viet Nam. After harvest, fruits were transported immediately to the laboratory where they were graded for uniform in colour and size (70-80 g) and hand-washed in chlorinated water (250 ppm), this was followed by the air-drying of fruits under fans.

Figure 1 Mangosteen using in the experiment

2.1.2 Packaging materials and preparations

Packaging materials used in the study were including polypropylene (PP) bags with a dimension (20x32 cm) and a thickness of 0.5 mm; MAP Lifespan (Lifespan) bags and perforated cardboard boxes (2.5 kg) were used as fruit containers for the experiment. To make micro-perforated PP bags for the experience, the polypropylene (PP) bags were punctured with a 0.33mm hypodermic needle to produce bags with 10, 20, 30 and 40 holes per bag (PP10, PP20, PP30, PP40 respectively).

2.2 Experimental design and treatments

This experiment was designed in a completely randomized design (CRD) with one factor of five bagging treatments including four treatments of micro-perforated polypropylene (PP) bags (i.e. PP10, PP20, PP30, PP40) and one of MAP Lifespan bag and a non-bagged control. The treatment of Lifespan bag was used here to compare the results of polypropylene (PP) bags with a commercial MAP (Lifespan) bag. All the treatments were replicated four times and each replication has12 fruits.
Harvested mangosteen fruits after graded, hand-washed then air-dried under fans as described as above, they were randomly divided into six groups (144 fruits/group) and packed in order in micro-perforated polypropylene (PP) and Lifespan bags (12 fruits/bag) and then the fruit bags were put individually into perforated cardboard boxes. For the control, fruits were just put into perforated cardboard boxes (12 fruits/cardboard box). The fruit boxes were then stored in a cool store with a temperature set at 13°C and relative humidity in the range of 80-85%. In each storage period of 10, 20 and 25 days, five fruit boxes containing all treatment and control were taken out from the coolstore and subjected to measure in-package gas composition which was followed by assessing physicochemical qualities of mangosteen fruit.

2.3 Studied physicochemical attributes of Mangosteen fruits

After completion storage periods, measurements were made for packaged gas composition and physicochemical assessments. Gas composition (Percentage of O2 and CO2) of packaging atmosphere was directly measured using Dansensor (Checkmate 3- Germany) once fruit boxes had been taken out from the coolstore. For physicochemical assessment, fruits were removed from their bags and held at a cool room (20°C) for 2 hours before the assessment had taken place. The physicochemical assessment was carried out on the basis of external and internal quality attributes of mangosteen fruit.

The selected external quality attributes were pericarp color, calyx and stem appearance, pericarp hardening and weight loss and they were non-destructively assessed from 144 fruits for each treatment at each measurement time including at the beginning, 10, 20 and 25 days of storage. After non-destructive assessment, the fruits were subjected to measure titratable acidity (TA) and total soluble solid (TSS) of fruit flesh. Among various physical characteristics, pericarp color was measured at or around the equator for each fruit by using a Minolta-CR400 chromameter (Japan) and the color was expressed as L* (lightness), a* (green to red color) and b* (blue to yellow).

Further, Calyx and stem appearance was assessed by visualization and based on the scoring rates given by Jiang & Li (2001). Calyx appearance was assessed on the 0-5 scale, where: 0 = no browning of calyx surface, 1 = 1-5% browning, 2 = 6-11% browning, 3 = 11-25% browning, 4 = 26-50% browning, 5 = > 50% browning and a browning index was calculated using the formula of Jiang & Li (2001):

\[ \sum \text{(browning value X percentage of fruit in each class)} \]

Further, stem appearance was also assessed by the 0-3 scale given by Jiang & Li (2001) where: 1 = green normal, 2 = yellow color, 3 = brown and black color and a browning index was similarly calculated as using the formula Jiang & Li (2001):

\[ \sum \text{(browning value x percentage of fruit in each class)} \]

Fruits having a browning index of calyx and stem appearance above 3.0 were rated as unacceptable.

Weight loss was expressed as percent weight loss which was calculated from initial weight and weight after storage using an electric balance (UX420S, Japan), according to the method given by Tefera et al. (2007).

Weight loss (%) = (Initial weight - weight at the assessment time) / initial weight x 100.

Further Pericarp hardening was expressed as the proportion of fruit with hardened pericarp, and it was calculated by the formula given below

Pericarp hardening (%) = (Number of fruit with hard pericarp / Total number of fruit in the treatment) X100.

Titratable acidity (TA) was determined by the method of the Association of Official Analytical Chemists International (1990) while the TSS was measured as degrees brix in fruit juice using an ATAGO-Japan, refractometer with a 0 - 32° scale. Analyses of TA and TSS were performed before storage and after 10, 20 and 25 days of storage.

2.3 Statistical analysis

All data were statistically analyzed by ANOVA and treatments compared using (to 5% significance level) by using SAS, version 8.1 and Excel 2010 software.

3 Results and Discussion

3.1 Effects of packaging materials on in-package gas composition

Effects of the different types of packaging materials on gas composition are shown in Fig.2. Results of study revealed a consistent decrease in concentration of O2 and simultaneous increases in the concentration of CO2 over the storage period for all packaging conditions. After 25 days of storage, the lowest O2 concentrations were reported from the Lifespan (5.29%) and PP10 (10.4%) packages and these two were significantly lower (p < 0.05) than the concentrations of the other treatments. The concentration of CO2 increased with storage time and it was reported highest in the treatments PP10 (10.5%) and Lifespan (7.4%) packages (p ≤ 0.05) after 25 days of storage. Results of this study revealed that both PP microperforated and Lifespan bags has ability to modify the in-package gas composition. Results of present study are in agreement with the findings of previous researchers those who reported effect of different packaging material on the gaseous concentration of mangosteen fruits (Pranamornkith et al., 2003; Pakkasarn et al., 2003; Manurakchinakorn et al., 2008). From the results of this study, it can be conclude that in-package gas composition (O2, CO2) can be successfully modified by using permeable packaging materials.
Further, modification in gaseous composition in-packaging condition also modify the storage atmosphere which affect the rate of respiration, ethylene production and the growth of postharvest pathogens and these conditions alter the physicochemical characteristics of the fruit (Suparlan & Kazuhiko, 2003; Xing et al., 2010).

3.2 Effects of different packaging materials on non-destructive quality attribute

3.2.1 Pericarp colour

Pericarp color is one of the most important visual attributes for mangosteen fruit quality and it is also a main criterion for maturity evaluation (Tongdee & Suwanagul, 1989). Changes in pericap color can be measured by chromameter with the help of three different colors and its related symbols viz L* (lightness), a* (green to red colour), b* (blue to yellow). Results of pericap color changes are shown in Table 1. Results of study suggested that L* values rapidly decreased for the first 10 days of storage and later on rate of color changes reduced at slower rate by the 20th and 25th days of packaging. After 25 days of storage, fruit stored in PP 30 had shown highest brightness but the differences among other treatments were not significant (P<0.05). All packaged fruit were significantly brighter (higher L* values) than the unpackaged control. Further, it was also reported that values of a* increases rapidly for the first 10 days of storage (except control) and later on the values of a* increased slowly for next 15 days (up to 25 days). Reductions in b* value also followed the same trends of L* values during storage. This reduction in L*and b* values and increases in a* values are correlated with color development of mangosteen due to ripening.

Table 1 Influence of packaging material with different numbers of holes and Lifespan bag on pericarp colour (L* (lightness), a* (green to red colour), b* (blue to yellow)) of mangosteen fruits during storage at 13°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>L* (Storage times (Days))</th>
<th>a* (Storage times (Days))</th>
<th>b* (Storage times (Days))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>PP10</td>
<td>55.70</td>
<td>41.99a</td>
<td>36.93*</td>
</tr>
<tr>
<td>PP20</td>
<td>54.57</td>
<td>35.92b</td>
<td>33.39b</td>
</tr>
<tr>
<td>PP30</td>
<td>52.96</td>
<td>39.54b</td>
<td>31.93b</td>
</tr>
<tr>
<td>PP40</td>
<td>58.78</td>
<td>35.16c</td>
<td>30.47b</td>
</tr>
<tr>
<td>Lifespan</td>
<td>53.64</td>
<td>41.28*</td>
<td>33.23b</td>
</tr>
<tr>
<td>Control</td>
<td>49.16</td>
<td>38.88b</td>
<td>37.59a</td>
</tr>
<tr>
<td>CV(%)</td>
<td>8.42</td>
<td>5.59</td>
<td>5.18</td>
</tr>
<tr>
<td>F</td>
<td>NS *</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Data shown in the table is mean values of four replicates; in the same column, values which have the same characteristics are not significant difference at (P<0.05); NS: Non significant difference at (P<0.05).
According to Noichinda (1992) mangosteen is a climacteric fruit and it is continuously ripen even after the harvesting. Further, Ratanamarno et al. (2005) reported that the development of pericarp color is the result of chlorophyll degradation and anthocyanin synthesis and in case of mangosteen fruits, rate of anthocyanin synthesis is maximum during fruit ripening and fruit become purple-red when it is completely ripen (Du & Francis, 1977). The fruit from all the packaged treatments continued to ripen during the three day shelf life storage period at 20°C. Fruit from the unpackaged control did not ripen well and at the end of the experiment retained a yellow skin color with scattered pink spots. Overall the best quality fruit at the end of the storage and shelf life period were those which stored in PP30 bags and these results are in agreement with the findings of Palapol et al. (2009).

3.2.2 Calyx characteristics

Changes in the appearance of the calyx and stem color during storage time are shown in Fig.2. The extent of browning and shrinkage of the calyx and stem increased with the increasing of storage time and significant differences was reported among the various treatments (p < 0.05). Rate of browning index increased with the storage time and among various tested treatments, lowest browning index (score above 1) was reported for the fruit stored in PP30 (score - 1.14) which was significantly lower than the control fruit which scored 2.41. After 25 days of storage only fruit stored in PP30 had as significantly lower (p < 0.05) browning index of the calyx (score = 2.84) than the other treatments (score 4-5) in which the calyx color turned to yellowish-brown (Figure 3a).

3.2.3 Stem characteristics

Storage time has visual effect on stem color and it gradually changes from green to yellow to brown. Among various tested conditions highest stem browning index was reported in non-packed fruit (score = 2.75). Chlorophyll discoloration in the calyx and stem highly influences consumer behavior of acceptance the fruit (Manurakchinakorn et al., 2008). Most acceptable stem color was maintained in fruit stored in PP30 which had a score of 2.53 (Figure 3b) and which was significantly lower than that of the other treatments. Color changes of calyx and stem could be related to the degradation of chlorophyll by chlorophyllase and water loss during the cold storage (Azuma et al., 1999; Manurakchinakorn et al., 2014).

3.2.4 Pericarp hardening percentage

Pericarp hardening in mangosteen greatly influences consumer behavior (Tongdee & Suwanagul, 1989; Ketsa & Koolpluksee, 1993; Deewatthanawong et al., 2003). Effects of packaging conditions on pericarp hardening are presented in Fig.4a. No pericarp hardening percentage (PHP) was observed in any treatment for the first 10 days of storage. After 10th days of storage PHP starting appearing stored mangosteen fruits and on 20th day highest PHP was detected in the control (16.67%) and it was followed by PP10 and Lifespan stored fruit. However, differences in PHP between packaged treatments were not found significant (p <0.05). After 25 days of storage, the PHPs of all bagged treatments were significantly lower (p <0.05) than the unpackaged control (44.4%). Among various treatments, fruit bagged in PP30 had lowest PHP (2.77%) but that value was not significantly lower than that of fruit in any of the other packaging treatments. Increases in peel hardness during cold storage could be the result of an increase in lignin and cross-linking between lignin, cell wall polysaccharides (Ralph et al., 1995) and protein (Whetten et al., 1998) creating solid lignin compounds (Iiyama et al., 1994). Results of this study are in agreement with data reported by Manurakchinakorn et al. (2008). Thus the use of appropriate packaging could inhibit pericarp hardening during cold storage.

Figure 3 Influence of of use PP micro holes to calyx (a) and stem colour changes (b) of mangosteen fruits during storage at 13°C.
3.2.5 Weight loss

Fruit weight loss is associated with the dehydration and it may affect the commercial values of the fruits. Effects of the different packaging materials on weight loss in present study are shown in Figure 3b. The percentage weight loss increased progressively with storage time in all the treatments. Unpackaged control fruit sustained an average of 15.45% weight loss. Weight loss was minimal (approximately 1%) in all packaged fruit and significantly lower (p<0.05) than the control fruit.

Among various tested packaging conditions, least weight loss was reported in fruit stored in the PP10 bags but this weight difference were not significant different in weight loss among the packaging treatments. These results are consistent with Daryono & Sabari (1986) and Cheohom, (1997) those who suggested that surface coatings and fruits wrapping in polyethylene film bags reduced weight loss during storage (Figure 4b).

3.3 Effects packaging materials on Total Soluble Solids (TSS) and Titratable Acidity (TA)

Effect of different packaging material on stored fruits TSS and TA have been sown in table 2. In general, very few changes was reported in TSS during storage, and it ranged from 15.7 to 18.6°Brix (table 2). Further, it was reported that TSS value increased slightly up to the 20th day of storage and later on it start decreasing towards the end of storage time. However, there were no significant differences reported among the means value of various treatments (p < 0.05). TSS values reported for this study were similar to reported by Augustin & Azudim (1986) who found that the TSS of mangosteen stored at 8 °C ranged from 17.7 and 20.4 °Brix.

In case of TA, some changes were reported in titratable acidity during storage in all treatments but these changes were not significantly different (p < 0.05) among the means of the six treatments. Further, slight reduction in TA value was reported after 20th day of storage and later on this value started increasing during the last five days. The average values ranged from 0.81% (day zero) to 0.78% (day 25) (table 2). The overall decrease in TA is attributable to the metabolism of organic acids and other chemical components by respiration and transpiration (Chitarra & Chitarra, 2005). These results are in agreement with the findings of Manurakchinhakorn et al. (2008) those who reported that during the ripening process TA decreased and TSS increased in most of the fruits.

Conclusions and Recommendations

The microperforated PP film used in this trial was just as effective as the commercial packaging product MAP Lifespan for controlling the in-package gas composition of stored mangosteen fruits. Among the various treatments, the PP bag with 30 holes was reported most effective at minimizing browning and shrinkage of calyx and stem, weight loss and pericarp hardening. It was also able to provide stability to biochemical components such as TSS and TA. The fruit were maintained in good physical and physiological condition for 25 days at 13°C.

This trial provides evidence that quality losses in mangosteen fruits during the low temperature storage can be avoided by packaging in microperforated polyethylene bags. It is recommended that a further series of trials comparative PP30 and MAP Lifespan should be carried out to ensure that there result obtained here for PP30 is consistent and is a reliable alternative to MAP Lifespan. Further research on combining the use of PP30 packaging with other treatments such as 1-mcp may lead to an even longer storage life of mangosteen fruits.

Figure 4 Influence of different packaging materials on pericap hardening (a) and weight loss (b) of mangosteen fruits during storage at 13°C.
Table 2 Influence of packaging material with different numbers of holes and Lifespan bags on total soluble solid (TSS) and total acidity (TA) of mangosteen fruits during storage at 13°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TSS (°Brix)</th>
<th>Total acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0D</td>
<td>10D</td>
</tr>
<tr>
<td>PP 10</td>
<td>15.70</td>
<td>17.23</td>
</tr>
<tr>
<td>PP 20</td>
<td>16.26</td>
<td>17.46</td>
</tr>
<tr>
<td>PP 30</td>
<td>16.16</td>
<td>17.03</td>
</tr>
<tr>
<td>PP 40</td>
<td>16.50</td>
<td>16.83</td>
</tr>
<tr>
<td>Lifespan</td>
<td>16.76</td>
<td>17.70</td>
</tr>
<tr>
<td>Control</td>
<td>16.50</td>
<td>17.13</td>
</tr>
<tr>
<td>CV(%)</td>
<td>3.65</td>
<td>3.70</td>
</tr>
</tbody>
</table>

Data shown in the table is mean values of four replicates; in the same column, values which have the same characteristics are not significant difference at (P<0.05); NS: Non significant difference at (P<0.05)

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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ANALYSIS OF PHENOLIC COMPOUNDS FOR DETERMINATION OF CAMBIUM DIFFERENTIATION AND TRACHEAL ELEMENTS IN OLIVE GRAFT COMBINATIONS

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ABSTRACT

The aim of this investigation was to evaluate the effect of phenolic compounds during differentiation of cambium and tracheal elements in olive cultivars ‘Ayvalik’, ‘Domat’, ‘Gemlik’, ‘Memecik’, ‘Nizip Yaglık’ and ‘Sari Ulak’. These cultivars were grafted onto one year-old cv. ‘Gemlik’ rootstock. This rootstock has been propagated by cuttings. According to the results of histological studies for two years; new cambium cells were initiated in first three months while the formation of vascular bundles and sclerenchyma cells were initiated in six months. Large numbers of undifferentiated parancymatic cells were also determined in both side grafts of the cultivars ‘Ayvalik’, ‘Domat’ and ‘Nizip Yaglık’ after 3, 6and 12 months of grafting. Further, High levels of 4 hydroxyphenylacetic acid, vanillic and ferulic acids content were determined in the scion of the cultivars Ayvalik, Nizip Yaglık and Domat. As a result, during recovery of graft zone, development of new cambium tissues, vascular connections and sclerenchyma tissues occurred imperfectly in ‘Ayvalik’, ‘Domat’ and ‘Nizip Yaglık’ cultivars and graft zones of these combinations were found weak. Moreover level of 4Hydroxyphenylacetic acid and Ferulic acidphenolic compounds had high concentration in scions of ‘Ayvalik’ and ‘Domat’, respectively. These results illustrated existence of problem in differentiation of cambium and vascular systems in graft interface of ‘Ayvalik’ and ‘Domat’ cultivars.

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1 Introduction

Olive is one of the most important crops all over the world, especially in Mediterranean region (Aguilera et al., 2005). It is widely cultivated for the production of olive oil and table olives. Grafting is one of the most common plant propagation methods in fruit trees for control the characteristics of scion growth (Tworkoski & Miller, 2007). This method is considerable in the adaptation of important cultivars in different areas. Successful formation of graft depends on various complexes of biochemical and structural procedures, which includes callus formation, establishment of new vascular tissue, and formation of an active vascular system across the graft interface (Hartmann et al., 1990; Errea et al., 2001; Pina & Errea, 2005).

Many researchers used different methods for determination of graft incompatibility, such as phenol analysis (DeCooman et al., 1996; Errea 1998; Musacchi et al., 2000; Usenik & Štampar, 2001; Usenik et al., 2006; Mng’omba et al., 2008), histological studies (Errea et al., 1994c; Ermel et al., 1995; Ermel et al., 1999; Mng’omba et al., 2007), isozyme analysis (Fernandez-Garcia et al., 2004; Gülen et al., 2005) and accumulation of carbohydrates (Moing & Gaudillere, 1992; Ciobotari et al., 2009).

According to Zucker (1983) phenolic compounds have an important role in determination of plants graft incompatibility especially with regard to ecological interactions. Further, Errea et al. (1994b) reported accumulation of two phenols in incompatible combinations in apricot. Effect of phenol compounds in incompatible combinations was reported by Treutter & Feucht (1986) in cherry trees (Prunus avium). Prunin and p-coumaryl-glucoside were found in the phloem of less compatible combinations of cherry (Prunus avium) on sour cherry (Prunus cerasus). Errea (1998) believed that quality and quantity of phenol patterns in rootstock-scion parts explained decreasing of metabolic functions at the graft union. Moreover, existences of various phenolic compounds have been signified procedures of division, development and differentiation into new tissues at the graft union.

Mng’omba et al. (2008) showed that high phenol concentration was obtained from less compatible combinations versus compatible combinations. High peaks obtained above the grafts union were r-coumaric acid. So, phenols especially r-coumaric acids and flavonoids caused poor callus formation at the union. This is the explicit sign of graft incompatibility. High percentage of lipids, phenols, small cell size and disorganized arrangement cells were observed in incompatible combinations (Errea et al., 2001). Accumulation of two phenols in incompatible combinations of apricot was monitored by high performance liquid chromatography (HPLC) and light microscopy (Errea et al., 1994b). Increase of phenylpropanoid metabolism in the incompatible unions result stress situations between scion-rootstock partners (Pina & Errea, 2009). It’s believed that a lot of phenolic compounds accumulate in wounded plants, which is main problem ingraft compatibility and specific phenols could cause graft incompatibility. The objective of this work was to determine the relationship between phenol compounds production and presence or absence of graft success in three major Turkey olive cultivars onto Gemlik cultivar at the graft zones and how the differentiation of cambium and tracheal elements was occurred in these cultivars.

2 Materials and Methods

2.1 Plant material

This investigation was carried out in 2011-2012 at Horticulture department of Agricultural, University of Ankara, Turkey. Olive cultivars ‘Ayvalik’, ‘Domat’, ‘Gemlik’, ‘Memecik’, ‘Nizip Yağlık’ and ‘Sari Ulak’ were obtained from Olive Research Institute, Bornova, Izmir, Turkey and were used as plant materials in this study. These plants were used as scions and were grafted (T-budding) onto one year-old ‘Gemlik’ cv. as rootstock. ‘Gemlik’ cv. was propagated by cuttings at Edremit Olive Nursery Station, Balikesir, Turkey. These grafts were maintained in the field for recording of grafting success of each sample.

2.2 Histological preparation

The samples collected from grafted plant were analyzed 3, 6 and 12 months after grafting. Three plants were used for each graft combination, at each stage of sampling for preparation sections. For each plant, one cm above and one cm below of the grafting region were used for analysis. Collected section were protected by using formalin aceto alcohol (FAA) solution (96% ethyl alcohol 900 ml; 5% glacial acetic acid 50 ml; 10% formaldehyde 50 ml; v/v/v). The fixed samples were sliced by using a microtome (Thermo Shandon Finesse 325), to obtain 30 µm-thick sections for microscopic operations (Leica EZ4D and Leica DM500 microscope). The cross sections were stained with safranin-fast green (60 seconds for each stain) and covered with a thin glass cover slip after addition of 10% glycerin (Espen et al., 2005).

2.3 Phenol extraction

One year old grafted plants of ‘Ayvalik’, ‘Domat’, ‘Gemlik’, ‘Memecik’, ‘Nizip Yağlık’ and ‘Sari Ulak’ on to ‘Gemlik’ cultivar were used for phenol extraction. Three plants were used for each graft combination. Bark of scion and rootstock of each plant were cut one cm above and one cm below of the graft. The bark samples that comprise the vascular cambium and the phloem were ground by using a mortar and pestle.
For phenol extraction, 0.50 g of fine powder was placed in Ependorf tubes and added 20 ml of methanol (60%) solution in tubes (Meirinhos et al., 2005). Samples were kept in dark conditions for 24 hours on shakers at room temperature. It was followed by storing this mixture at 4°C and centrifuged 8000 rpm for 10 min by a bench centrifuge (Sigma 3K30). This procedure repeated two times with 20 and 10 ml methanol (60%) solution respectively. Final obtained volume of extraction was 50 ml. After mixing of all the supernatants, 10 ml was taken for total soluble phenol qualification.

2.4 HPLC-high performance liquid chromatography

Separation of different phenolic compounds was carried out on a Shimadzu HPLC (LC-10A) system with diode array detector, Thermo (250 mm x 4.5 mm) column. The mobile phase consisted of solvent A: 100% methanol and solvent B: 2% acetic acid. Gradient was linear from 0% to 90% of solvent B and its duration was 60 min, by flow rate of 1 ml/min.

Caffeic, Ferulic, ρ-coumaric, Vanillic acids, Quersetin, Quersetin 3-β-D Glucoside, Rutin trihydrate and 4-Hydroxyphenylacetic acid were individual phenol compounds as standards (Meirinhos et al., 2005).

2.5 Statistical analysis

Experimental design for phenol analysis was randomized complete block design with three replications. Statistical analysis was done with the IBM SPSS Statistics Version 21 Software.

3 Results and Discussion

3.1 Histologic analysis

Callus formation in all graft combinations mainly were take placed in the both side of grafts, and no differences have been recorded between any combinations. Formed Callus tissue, that fills space between scion and rootstock, is the essential stage for development of future cambium and vascular systems at graft interface (Errea et al., 1994a; Wang & Kollmann, 1996). When ‘Gemlik’, ‘Memecik’ and ‘Sari Ulak’ cultivars grafted on ‘Gemlik’ rootstock, in both years, 3 months after grafting in wide range the callus tissue and subsequent cambial zone formation have been occurred and vascular tissue were differentiated. Moreover sclerenchyma tissues were identified in ‘Sari Ulak’ on ‘Gemlik’ graft. Fontanazza & Rugini (1983) showed that new vascular tissue formed during 3 months after grafting, also after 4 months of grafting sclerenchyma ring at graft union start appearing. But in ‘Ayvalik’, ‘Domat’ and ‘Nizip Yaglık’ cultivars grafted onto ‘Gemlik’ rootstock, cambial zone formation were occurred in very restricted spaces (Figure 1).

After 6 and 12 months of grafting, widely undifferentiated parenchymateous cells were observed at both sides of ‘Ayvalik’, ‘Domat’ and ‘Nizip Yaglık’ cultivars grafts. Errea et al. (1994a) reported the presence of parenchymatic tissue in weak combination of incompatible Prunus species. Mng’omba et al. (2007) described existence of undifferentiated tissues (parenchymal cells) in the incompatible graft combinations of Uapaca kirkiana Müell Arg. Results showed that these parenchymateous cells were not determined or was very thin layers at both sides of grafts of ‘Memecik’ and ‘Sari Ulak’ cultivars grafted on ‘Gemlik’ rootstock. Differentiations of cambium cells were developed in ‘Gemlik’, ‘Memecik’ and ‘Sari Ulak’ cultivars on the ‘Gemlik’ rootstock. Although cambium cells were discontinues and scattered form, in graft zone, but these differentiated tissues were determined in ‘Ayvalik’, ‘Domat’ and ‘Nizip Yaglık’ cultivars combinations. Hartmann et al. (1990),mentioned that new cambial cells, derived from the newly formed callus, are differentiated and formed as a continuous cambial connection between rootstock and scion.

Figure 1 Three months old of budded graft of Ayvalik cultivar onto Gemlik rootstock (‘The photograph was taken by bright field light microscopy. The C. shows callus; S. scion; R. rootstock’)
In the final step, formation of tracheal elements from new cambium cells is very important. These new differentiation tissues permit translocation of soluble material between the stock and the scion. Results showed that tracheal elements and sclerenchyma tissues were formed in graft union of ‘Gemlik’, ‘Memecik’ and ‘Sari Ulak’ cultivars onto ‘Gemlik’ rootstock but discontinuity of cambium cells leads to interruption of tracheal elements in ‘Ayvalik’, ‘Domat’ and ‘Nizip Yaglik’ cultivars combinations (Figure 2). Most researchers believed that a graft union can be considered successful and complete when several vascular connections exist on the graft interface (Moore, 1984; Simons, 1987).

Also the establishment of vascular connections and their function is principal step in determining the compatibility of graft combinations (Gebhardt & Goldbach, 1988). Likewise Ermel et al. (1997) showed that cell necrosis and lack of vascular connection continuity in graft union was the manifest symptoms of graft incompatibility. Errea et al. (1994a) reported that insufficient differentiation of callus cells in some areas of graft union affected the newly formed xylem and phloem activity. Results of this study support the view that said the formation of vascular connection is not a guarantee of a successful development union in grafted olive plants in this study.

3.2 Phenol Analysis

The presence of phenols accumulation at the union serves as an indicator of problems in grafting combinations. Stains of graft union with glutaraldehyde (%3) and osmium tetroxide-KI, confirmed the existence of phenolic compounds in all tissues of grafts unions (Figure 3). For this reason, in the first step content of phenolic compounds were determined above and below of graft union. Results of this study revealed the presence of phenolic compounds such as 4 hydroxyphenylacetic, vanillic acid, ferulic acid and routine trihydrate content from the scions of grafts and this concentration was significantly different among the treatments (Table 1). Scions of Ayvalik had the highest level of 4hydroxyphenylacetic acid (46.036 mg/l). Moreover concentration of ferulic acid was reported higher in Ayvalik and Domat scionsthan Gemlik, Memeci k and Sari Ulak onto Gemlik rootstock. In callus cultures of Prunus avium, results showed that a high level of prunin limits the proliferation and differentiation of the cells (Feucht et al., 1988).

Table 1 Mean comparison of some phenolic compounds.

<table>
<thead>
<tr>
<th>Phenols Scions</th>
<th>4 Hydroxyphenylacetic acid (mg*l)</th>
<th>Vanillic acid (mg*l)</th>
<th>Cafeic acid (mg*l)</th>
<th>p -coumaric acid (mg*l)</th>
<th>Ferulic acid (mg*l)</th>
<th>Rutin trihydrate (mg*l)</th>
<th>Quersetin (mg*l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayvalik</td>
<td>46.036a</td>
<td>3.379c</td>
<td>0.9022a</td>
<td>10.924a</td>
<td>12.787b</td>
<td>31.653ab</td>
<td></td>
</tr>
<tr>
<td>Domat</td>
<td>0.000</td>
<td>14.395b</td>
<td>0.1800</td>
<td>22.158b</td>
<td>10.691a</td>
<td>4.236c</td>
<td>42.665a</td>
</tr>
<tr>
<td>Gemlik</td>
<td>1.256b</td>
<td>14.953b</td>
<td>0.8507a</td>
<td>15.482b</td>
<td>2.863b</td>
<td>34.799a</td>
<td>24.464a</td>
</tr>
<tr>
<td>Memecik</td>
<td>0.000</td>
<td>0.876a</td>
<td>0.6758b</td>
<td>20.492a</td>
<td>1.656b</td>
<td>12.068a</td>
<td>31.233ab</td>
</tr>
<tr>
<td>Nizip Yaglik</td>
<td>0.000</td>
<td>28.173b</td>
<td>0.7841a</td>
<td>25.059a</td>
<td>2.387b</td>
<td>4.088c</td>
<td>39.572ab</td>
</tr>
<tr>
<td>Sari Ulak</td>
<td>0.000</td>
<td>31.970a</td>
<td>0.7204a</td>
<td>23.657a</td>
<td>4.757b</td>
<td>4.873c</td>
<td>32.868ab</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at 0.05.

Figure 2 Six months old of budded graft of Domat cultivar onto Gemlik rootstock. (“The photograph was taken by bright field light microscopy. The C. shows callus; S. scion; R. rootstock”)

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De Cooman et al. (1996) reported that ρ-coumaric acid accumulation in graft zone of incompatible combinations of Eucalyptus gunnii. Usenik et al. (2006) reported high level of ρ-coumaric acid accumulation in the upper part of the graft union in incompatible combination of apricot. Also Mng’omba et al. (2008) observed high level of phenolic compounds accumulation and necrotic lines in Uapaca kirkiana Müell Arg. incompatible combination streak. May be, Existence of high concentration of these phenolic compounds in scions of graft combinations informs as reason of cambium tissues differentiation inhibitors.

**Conclusion**

Results of study revealed that cambium cell formation and differentiation were slow in Ayvalik, Domat and Nizip Yaglik cultivars grafted on Gemlik rootstock as compared to Memecikand Sari Ulak cultivars. Furthermore, discontinuity of cambium cells leads to interruption of tracheal elements. Accordingly, differentiation of callus tissues on cambium and vascular elements had problem in graft interface of these cultivars. Moreover numbers of phenolic compounds such as 4-Hydroxyphenylacetic acid and Ferulic acid had high concentration in scions of ‘Ayvalik’ and ‘Domat’, respectively. These results demonstrated existence of problem in dedifferentiated of cambium and vascular systems in graft interface of ‘Ayvalik’ and ‘Domat’ cultivars.

**Acknowledgements**

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**Conflict of interest**

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

**References**


Tworkoski T, Miller S (2007) Rootstock effect on growth of apple scions with different growth habits. Scientia Horticulturae 111 : 335–343


ABSTRACT

Present study was carried out to evaluate the effects of irrigation regimes and zeolite on the yield and quality parameters of silage corn hybrids. Study was conducted at Seed and Plant Improvement Institute in Karaj, Iran during 2013. The experiment was arranged in a three-replicated split-split plot based on randomized complete blocks design (RCBD) including three irrigation regimes as main plots (irrigation after 70, 100 and 130 mm evaporation from standard class A evaporation pan), two levels of zeolite as sub plots (0, 10 ton/ha) and three hybrids of silage corn (KSC704, KSC705 and KSC720) as sub sub plot. Results of this study indicated that increasing water stress from optimum irrigation (Ir70) to moderate (Ir100) and low irrigation (Ir130) caused 25 and 37% reduction in forage dry matter yield but no significant difference was reported in protein yield. Moreover, application of zeolite have significant effect on forage yield and also have significant effect on protein yield (P<0.01). Forage quality parameters including water soluble carbohydrates, crude protein, acid detergent fiber and neutral detergent fiber increased and dry matter digestibility reduced when there was limited irrigation imposition. Among the tested corn seed hybrids KSC704 hybrid produced the highest dry forage yield and in terms of protein yield, no significant difference was reported between KSC720. KSC705 hybrid had the lowest acid detergent fiber and neutral detergent fiber and the highest dry matter digestibility.
1 Introduction

In present scenario drought became a worldwide problem and most of the applied researches are based on the drought resistance sustainable crop production (Kojić et al., 2012). Demand of water for irrigation continuously increasing while a drastic reduction was reported in the availability of water, condition became more critical under arid and semi-arid environmental conditions (Rostamza et al., 2011). Alternative forage sources which can grow under these extreme conditions could be utilized to cope with the declining water availability (Marsalis & Bean, 2010). Approximately, seventy three (73) percent of Iranian agricultural production area has arid and semi-arid climatic conditions (Abarghouei et al., 2011). Therefore, effective alternative management strategies are required for the efficient uses of water. Among the available information, retaining soil moisture and preventing substantial loss of water by the use of zeolite was used by researchers (Mumpton, 1999; Armandpisheh et al., 2009; Ahmed et al., 2010). Zeolites are hydrated aluminosilicates of alkaline with open three-dimensional structure and are able to lose or gain water reversibly and exchange extra framework cations, both without crystal structure changes (Mumpton, 1999). Zeolites can act as water moderators and can absorb up to 55% water of their own weight, later on this water released slowly as per plant water demand (Pisarovic et al., 2003).

Further, Ahmed et al. (2010) and Polat et al. (2004) also reported that Zeolites can enhance the better utilization of NPK nutrition and play an important role in the plant growth. Krutilina et al. (2000) also reported a significant effect of zeolite on the improvement of biomass production and rate of photosynthesis in maize and barley crops. Similarly, Keshavaz & Farahbakhsh, (2012) reported that under the drought conditions application of zeolite increased the yield of millet crop. Also, similar type of improvement was reported in rapeseed (Valadabadi et al., 2010) and Lathyrus sativus (Pirzad & Mohammadzade, 2014).

Forage crops play an important role in supplying energy and protein to livestock (Eskandari et al., 2009). In breeding of forage crops, increase of yield and forage quality are the main factors which play prominent role in the introduction of new varieties. Forages with good quality should have high dry matter yield, energy, digestibility and low fiber for optimal fermentation in the silo and storage. Silage corn have almost all these features, except the high protein content and this phenomenon make it better than other forage plants (Curran & Posch, 1999). Various researchers reported the scarcity of irrigation water may cause reduction in dry matter yield of forage crops (Vasilakoglou et al., 2011; Keshavaz & Farahbakhsh, 2012; Jahanzad et al., 2013). Knowledge about the influence of drought on nutritive quality of forage plants is inconsistent and limited. Some studied like Jahanzad et al. (2013) reported that drought condition may cause reduction in NDF and ADF concentration while it can improve the concentration of CP, DMD, WSC and DMD in forage sorghum cultivars. Similar type of findings was reported by Newman (2014) under drought stress conditions. Further, Xu & Lascano (2007) reported that drought stress reduced the silo quality.

Compared to well-watered treatments, the silage produced from drought stressed plants had higher CP, ADF, NDF, lignin contents and lower total digestible nutrients and starch. Limited informations are available with concern to the integrated effects of irrigation and application of zeolite on yield and forage quality of silage corn. KSC704 is currently the most common hybrid of silage corn in Iran. However, newly released hybrids, KSC720 and KSC705, have been also introduced to eliminate the probable problems concerning with a single corn planting in the country. The objective of this study was to determine the effect of zeolite on yield and quality of three silage corn hybrids (KSC704, KSC705 and KSC720) under normal and stress conditions.

2 Materials and Methods

The experiment was conducted at the experimental field of Seed and Plant Improvement Institute, Karaj, Iran (35°47’N, 50° 55’ W, altitude 1254.5 m) during 2013. Study area has an average of 150-160 dry days in a year and coming in the hot and dry Mediterranean climate zones. The experiment was arranged in split-split-plot with three replicates by following randomized complete blocks (RCBD) design. Three irrigation regimes including Ir N (optimum irrigation -irrigation when evaporation reached 70 mm, using evaporation pan class “A”), Ir M (moderate) and Ir L (low irrigation) were used as main plots. While, two levels of zeolite (0, 10 ton/ha) were used as sub-plots and also three hybrids of silage corn (KSC704, KSC705 and KSC720) were considered as sub-sub-plots.

The experiments were carried out in a clay-loam soil. Important physical and chemical properties of soil based on soil test results are presented in Table 1 and on the basis of these results dose of fertilizers application were determined. To determine the physical and chemical properties of soil at experimental field, 15 soil samples were randomly collected from 0-30 and 30-60 cm depth. Samples were carefully mixed together and turned into a single sample and were transferred to the laboratory. Physicochemical properties of the collected soil samples were determined by method described by Hesse (1971) and have been shown in Table 1. Distance between rows and plants per row were considered 75 and 15.5 cm, respectively (85000 plants. ha⁻¹). Each sub sub-plot included 4 rows with spacing of 0.75 m and length of 6 m. Irrigation regimes were applied when plants were completely established with 6 to 8 leaves, by average. Plots were irrigated when evaporation reached the considered amount for each irrigation regimes (70, 100, and 130 mm evaporation from the surface of the class A evaporation pan). In order to determine the water volume for irrigation, a soil sample was collected from each plot in depth of root development region before irrigation.
The samples were kept in 80°C oven for 24 hours. The weight of soil moisture content was determined by the method given by Azizi & Mahrokh, 2012 where moisture content was calculated by using equations 1 and the volume of water needed for irrigation was determined by using equations 2 and 3.

Moisture content in soil (Өm) = wet soil weight (gr) - dry soil weight (gr)/ dry soil weight( gr) ………………….1

\[ H = \rho_b (\Theta F.C - \Theta m) D \] …………………….2

\[ V = H \times A \] …………………….3

Where \( H \) is water height in the plot; \( \rho_b \) is soil bulk density; \( \Theta F.C \) is the moisture level at field capacity; \( \Theta m \) is plot moisture at irrigation time; \( D \) is the root development depth in different growth stages and \( V \) is the irrigation water volume needed for each plot and \( A \) is the plot area (18 m\(^2\)).

10 plants were randomly selected from each plot to determine dry forage yield. Then, the samples were dried in an oven at 70°C for 72 h, and dry matter yield per unit area was measured. Forage quality parameters was measured including crude protein (CP), water soluble carbohydrate (WSC), acid detergent fiber (ADF), neutral detergent fiber (NDF), dry matter digestibility (DMD) and ASH. Near infrared reflectance spectroscopy (NIRS) method described by Jafari et al (2003) was used for quality analysis. Dried samples were ground through the 0.1 mm screen of a cyclone mill and scanned using a near-infrared reflectance spectroscopy (NIRS, Informatics Perten 8600 Feed Analyzer) at wavelengths ranging from 500 to 2400 nm. Protein yield was calculated by multiplying dry matter yield to crude protein content and all values are given in % on dry matter basis. Data were analyzed using the analysis of variance (ANOVA) and general linear model (GLM) procedures of SAS (SAS Institute, 2003). Effects were considered significant at \( P \)-values ≤0.05 in the F-test. Duncan multiple range test was conducted for comparison of means.

3 Results and Discussion

3.1 Forage dry matter

Dry matter yield was significantly affected (\( P < 0.01 \)) by different irrigation regimes (Table 2), among various tested irrigation regimes, highest dry matter yield (15939 kg. ha\(^{-1}\)) was obtained from normal irrigation regime (Ir\(_{30}\)) while the lowest dry forage yield (10036 kg. ha\(^{-1}\)) was observed at low irrigation level (Ir\(_{130}\)). Irrigation regime, Ir\(_{30}\) has shorter irrigation intervals than Ir\(_{100}\) and Ir\(_{130}\) and it produced 25 and 37% higher forage dry matter than the rest two, respectively. Kramer & Boyer (1995) suggested that when soil water ratio is not enough to facilitate nutrient uptake by roots, plants face difficulty in absorbing essential elements such as nitrogen and phosphorus for their normal growth which caused reduction in final yield. This statement justified the results obtained in present study. Further, various researchers reported that impaired mitosis, cell elongation and expansion result reduced plant height, leaf area and crop growth under drought stress (Nonami, 1998; Kay et al., 2006; Hussain et al., 2008).

Now, in these days it is well established that reduced dry matter yield of forage is associated with drought stress (Marsalis et al., 2009; Marsalis & Bean, 2010; Rostamza et al., 2011; Jahanzad et al., 2013). Result of this study suggested that use of zeolite increased dry forage yield (\( P < 0.01 \)) by 20% from 11202 kg. ha\(^{-1}\) to 14106 kg. ha\(^{-1}\) (Table 3). According to Torkashvand & Shadparvar (2013) application of zeolite could be beneficial with respect to increased water holding capacity of soil. Similarly, Ahmed et al. (2010) suggested that zeolite application not only increase the uptake of N,P,K but also increase the efficiency of their use by plants. Valadabadi et al. (2010) reported significant improvement in the yield of rapeseed by the application of Zeolite under drought stress. Similar type of improvement was reported by Najafinezhad et al. (2014) in corn crop. These results are in accordance with the findings of present study. While Turk et al. (2006) reported contradictory results when they tested the effect of Zeolite on the yield of Alfalfa crops. Corn hybrids also differed significantly (\( P < 0.01 \)) in terms of forage dry matter (Table 2) and corn hybrid KSC704 produced the highest dry forage yield (14330 kg. ha\(^{-1}\)) and contained 10 and 25% higher forage dry matter than KSC720 and KSC705, respectively (Table 3). Interaction between various treatments has no significant effect on dry forage yield.

3.2 Forage quality

3.2.1 Crude protein

Crude protein content which is one of the most important factors in forage quality (Wang & Frei, 2011) has been significantly affected (\( P < 0.01 \)) by irrigation levels, zeolite application and corn cultivars (Table 2). In present study it was reported that less irrigation led to a progressive rise in CP content.
Various researchers reported that water deficit intensifies forage CP content as a result of nitrogen accumulation (Pessarakli et al., 2005; Haberle et al., 2008) and accumulation of protein metabolites such as proline in leaves (Pelleschi et al., 1997). Further, the negative effects of drought stress on dry matter accumulation have been suggested by many researchers as a main factor related to higher protein concentrations (Gooding et al., 2003; Asseng & Milroy, 2006; Weightman et al., 2008).

These results are in agreement with the findings of this study. However, Dwivedi et al. (1996) have reported lower protein concentration under drought stress. Significant interactions between irrigation levels and zeolite application were also reported in this study (P < 0.05). Zeolite reduced the negative effect of drought stress in mild (Ir00) and severe stress (Ir30) condition and increase the crude protein content but this improvement in crude protein content are not significantly differ that the normal irrigation condition (Ir00) (Table 4). Huang & Petrovic(1994) suggested that zeolite can improve the water retention capacity of the soil and decreased severity of drought stress, in this manner findings of this study are in agreement with these researchers. The hybrids also had significant differences (P < 0.01) in crude protein content (Table 1). At corn hybrid level, highest CP content (9.4%) was observed in KSC720 hybrid (Table 3).

Table 2: Analysis of variance of silage corn hybrids traits under irrigation treatments and zeolite.

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>df</th>
<th>Yield (kg ha⁻¹)</th>
<th>WSC (%)</th>
<th>CP (%)</th>
<th>Protein yield (kg ha⁻¹)</th>
<th>ADF (%)</th>
<th>NDF (%)</th>
<th>DMD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>53744014</td>
<td>0.675²</td>
<td>0.208²</td>
<td>380159²</td>
<td>8.81²</td>
<td>14.28²</td>
<td>3.58²</td>
</tr>
<tr>
<td>Irrigation</td>
<td>2</td>
<td>162850877</td>
<td>120.21²</td>
<td>41.35²</td>
<td>65994²</td>
<td>249.85²</td>
<td>586.58²</td>
<td>402.3²</td>
</tr>
<tr>
<td>Zeolite</td>
<td>7</td>
<td>13822281</td>
<td>17.17²</td>
<td>15.01²</td>
<td>245522²</td>
<td>10.15²</td>
<td>187.60²</td>
<td>14.23²</td>
</tr>
<tr>
<td>Irrigation*zeolite</td>
<td>1</td>
<td>319077²</td>
<td>8.229²</td>
<td>2.51²</td>
<td>18114²</td>
<td>20.54²</td>
<td>30.82²</td>
<td>6.49²</td>
</tr>
<tr>
<td>E(b)</td>
<td>6</td>
<td>490398</td>
<td>6.36</td>
<td>0.372</td>
<td>7628</td>
<td>6.25</td>
<td>37.39</td>
<td>6.33</td>
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<td>Hybrid</td>
<td>2</td>
<td>58719385</td>
<td>85.05²</td>
<td>11.64²</td>
<td>918926²</td>
<td>23.95²</td>
<td>82.18²</td>
<td>120.0²</td>
</tr>
<tr>
<td>Irrigation*Hybrid</td>
<td>4</td>
<td>597206²</td>
<td>6.95</td>
<td>0.473²</td>
<td>17387²</td>
<td>13.29²</td>
<td>35.34²</td>
<td>9.25²</td>
</tr>
<tr>
<td>Zeolite*Hybrid</td>
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<td>447291²</td>
<td>4.05²</td>
<td>0.0222²</td>
<td>5483²</td>
<td>0.28²</td>
<td>12.51²</td>
<td>6.93²</td>
</tr>
<tr>
<td>Irrigation<em>Zeolite</em>Hybrid</td>
<td>4</td>
<td>752988²</td>
<td>2.67²</td>
<td>0.132²</td>
<td>4403²</td>
<td>4.93²</td>
<td>10.81²</td>
<td>6.54²</td>
</tr>
<tr>
<td>E(c)</td>
<td>24</td>
<td>2797962</td>
<td>1.403</td>
<td>0.215</td>
<td>30937</td>
<td>5.56</td>
<td>10.90</td>
<td>4.49</td>
</tr>
<tr>
<td>C.V</td>
<td>-</td>
<td>13.21</td>
<td>4.35</td>
<td>5.37</td>
<td>16.59</td>
<td>9.10</td>
<td>7.44</td>
<td>3.17</td>
</tr>
</tbody>
</table>

ns - Non significant, * and ** significant at P<0.05 and P<0.01, respectively.

Table 3: Effect of irrigation regime, zeolite and hybrid on yield and quality traits of silage corn.

<table>
<thead>
<tr>
<th>Forage quality parameters</th>
<th>Yield (kg ha⁻¹)</th>
<th>WSC (%)</th>
<th>CP (%)</th>
<th>Protein yield (kg ha⁻¹)</th>
<th>ADF (%)</th>
<th>NDF (%)</th>
<th>DMD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation regime</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ir00</td>
<td>15939a</td>
<td>24.8a</td>
<td>7.0a</td>
<td>1120a</td>
<td>39.8a</td>
<td>22.1a</td>
<td>71.3a</td>
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<td>Ir100</td>
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<td>26.9b</td>
<td>8.9b</td>
<td>1062b</td>
<td>42.4b</td>
<td>26.0b</td>
<td>66.7b</td>
</tr>
<tr>
<td>Ir30</td>
<td>10036c</td>
<td>29.9c</td>
<td>10.0c</td>
<td>999c</td>
<td>50.7c</td>
<td>29.5c</td>
<td>61.9c</td>
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<tr>
<td>L.S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Zeolite</td>
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<td></td>
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<td></td>
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<tr>
<td>Z0</td>
<td>11020b</td>
<td>27.8b</td>
<td>9.1b</td>
<td>993b</td>
<td>42.5b</td>
<td>25.4b</td>
<td>67.1b</td>
</tr>
<tr>
<td>Z1</td>
<td>14330c</td>
<td>26.7c</td>
<td>8.1c</td>
<td>1128c</td>
<td>46.2c</td>
<td>26.3c</td>
<td>66.1c</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
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<tr>
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<tr>
<td>KSC704</td>
<td>14330c</td>
<td>29.3c</td>
<td>8.7c</td>
<td>1216c</td>
<td>45.0c</td>
<td>26.7c</td>
<td>65.7c</td>
</tr>
<tr>
<td>KSC705</td>
<td>10741c</td>
<td>25.0c</td>
<td>7.8c</td>
<td>801b</td>
<td>41.9b</td>
<td>24.6b</td>
<td>69.5b</td>
</tr>
<tr>
<td>KSC720</td>
<td>12891b</td>
<td>27.4b</td>
<td>9.4b</td>
<td>1164c</td>
<td>46.1c</td>
<td>26.4c</td>
<td>64.6b</td>
</tr>
</tbody>
</table>

Means in the same column followed by letters differ significantly at P < 0.05; Ir00, Ir100, and Ir30 represent high, moderate, and low irrigation levels; Z0 and Z1 represent consumption 0 and 10 ton per hectare, respectively; L.S.: level of significance; * P < 0.05; ** P < 0.01; Non-significant (ns) effect of interaction.

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Table 4 Effects of irrigation regime and zeolite interaction on yield and quality traits of silage corn.

<table>
<thead>
<tr>
<th>Irrigation regime</th>
<th>Zeolite</th>
<th>Yield (kg ha(^{-1}))</th>
<th>CP (%)</th>
<th>Protein yield (kg ha(^{-1}))</th>
<th>WSC (%)</th>
<th>DMD (%)</th>
<th>NDF (%)</th>
<th>ADF (%)</th>
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<tbody>
<tr>
<td>Ir(_{70})</td>
<td>Z(_0)</td>
<td>14640(^{b})</td>
<td>7.1(^{d})</td>
<td>1052(^{a})</td>
<td>72.5(^{c})</td>
<td>71.7(^{a})</td>
<td>39.1(^{a})</td>
<td>20.4(^{a})</td>
</tr>
<tr>
<td></td>
<td>Z(_1)</td>
<td>17238(^{c})</td>
<td>6.8(^{d})</td>
<td>1187(^{b})</td>
<td>72.4(^{c})</td>
<td>71.0(^{b})</td>
<td>40.5(^{a})</td>
<td>23.8(^{ab})</td>
</tr>
<tr>
<td>Ir(_{100})</td>
<td>Z(_0)</td>
<td>10442(^{e})</td>
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<td>1026(^{ab})</td>
<td>72.2(^{f})</td>
<td>65.7(^{c})</td>
<td>40.8(^{bc})</td>
<td>26.2(^{e})</td>
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</table>

Means in the same column followed by letters differ significantly at P < 0.05; Ir\(_{70}\), Ir\(_{100}\), and Ir\(_{130}\) represent high, moderate, and low irrigation levels; Z\(_0\) and Z\(_1\) represent consumption 0 and 10 ton per hectare, respectively; L.S.: level of significance; * P < 0.05; ** P < 0.01; Non-significant (ns) effect of interaction.

Similar types of findings was reported by Jahanzad et al. (2013) and Asay et al. (2002), these researchers reported that drought stress reduced dry matter yield and increased protein content in sorghum forage and tall fescue, respectively. However in their study the reduction amount of dry matter was very high and finally the protein yield reduced significantly when limited irrigation was imposed. Although zeolite did not have any significant effect on crude protein content, here also application of zeolite caused some increases in protein yield (P < 0.01) but also did not show any significant difference (Table 2). Similar, type of findings was Najafinezhad et al. (2014) and Nasri et al. (2012) reported by using zeolite, these researchers find some increase in protein yield of maize and sorghum. Hybrids had significant differences in crude protein content (P < 0.01) and KSC720 shows superiority in terms of protein yield on KSC704 and KSC705 (Table 3).

3.2.3 Water-soluble carbohydrates

Water soluble carbohydrates represent the most important source of energy in the finished diet (Coleman & Moore, 2003) and have positive influence on fodder intake and are important for an efficient utilization of dietary N (Küchenstein et al., 2013). WSC content was significantly (P < 0.01) affected by irrigation levels and cultivars as shown in Table 2. In this experiment, less irrigation led to a progressive rise in WSC content (Table 3). Significant increases in WSC content under drought stress may be because of osmotic adjustments of plants (DaCosta & Huang, 2006; Nakayama et al., 2007), reduction in starch formation (Bethke et al., 2009) and due to the inhibition in the activity of the enzyme starch synthase which resulting in impeded conversion of sugars to starch (Wang & Frei, 2011).

According to Buxton (1996) rate of photosynthesis usually less affected by drought as compared to the rate of respiration and growth and this may be a possible reason of increasing digestible soluble sugars in plants. Hybrids had significant difference (P < 0.01) on WSC content (Table 2). Among three hybrids, highest WSC content was observed in KSC704 hybrid (Table 3). Also, WSC content was influenced (P < 0.01) by the interaction of irrigation regime and hybrid (Table 2).

Table 5 Effects of irrigation regime and hybrid interaction on yield and quality traits of silage corn.

<table>
<thead>
<tr>
<th>Irrigation regime</th>
<th>Hybrid</th>
<th>Yield (kg ha(^{-1}))</th>
<th>CP (%)</th>
<th>Protein yield (kg ha(^{-1}))</th>
<th>WSC (%)</th>
<th>NDF (%)</th>
<th>ADF (%)</th>
<th>DMD (%)</th>
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<td>Ir(_{70})</td>
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<td>77.3(^{d})</td>
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<td>13828(^{a})</td>
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<td>873(^{ab})</td>
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<td>1212(^{ab})</td>
<td>72.4(^{d})</td>
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<td>1189(^{ab})</td>
<td>72.4(^{d})</td>
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<td>31.6(^{bc})</td>
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<td>759(^{b})</td>
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<td>66.1(^{d})</td>
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</tbody>
</table>

Means in the same column followed by letters differ significantly at P < 0.05; Ir\(_{70}\), Ir\(_{100}\), and Ir\(_{130}\) represent high, moderate, and low irrigation levels; Z\(_0\) and Z\(_1\) represent consumption 0 and 10 ton per hectare, respectively; L.S.: level of significance; * P < 0.05; ** P < 0.01; Non-significant (ns) effect of interaction.
Table 6 Effects of zeolite and hybrid interaction on yield and quality traits of silage corn.

<table>
<thead>
<tr>
<th>Zeolite</th>
<th>Hybrid</th>
<th>Yield (kg ha(^{-1}))</th>
<th>CP (%)</th>
<th>Protein yield</th>
<th>WSC (%)</th>
<th>NDF (%)</th>
<th>ADF (%)</th>
<th>DMD (%)</th>
</tr>
</thead>
<tbody>
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<td>KSC704</td>
<td>12753(^c)</td>
<td>9.2(^a)</td>
<td>1154(^b)</td>
<td>30.0(^a)</td>
<td>44.0(^b)</td>
<td>26.4(^a)</td>
<td>66.3(^c)</td>
</tr>
<tr>
<td>KSC705</td>
<td>9467(^b)</td>
<td>11384(^c)</td>
<td>9.9(^a)</td>
<td>1080(^a)</td>
<td>28.3(^b)</td>
<td>43.5(^b)</td>
<td>25.9(^b)</td>
<td>64.5(^b)</td>
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<td>1286(^b)</td>
<td>8.2(^a)</td>
<td>580(^a)</td>
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<td>25.9(^b)</td>
<td>64.5(^b)</td>
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</tbody>
</table>

Means in the same column followed by letters differ significantly at P < 0.05; Ir\(_{50}\), Ir\(_{100}\), and Ir\(_{130}\) represent high, moderate, and low irrigation levels; Z\(_0\) and Z\(_1\) represent consumption 0 and 10 ton per hectare, respectively; L.S.: level of significance; * P < 0.05; ** P < 0.01; Non-significant (ns) effect of interaction.

While in hybrids KSC704 and KSC720, mild stress increased the WSC content significantly, but in hybrid KSC705 the mild stress did not have significant effect on water-soluble carbohydrates. In combination also, highest WSC content (31.4%) was observed in the combination of Ir\(_{130}\) and KSC704 hybrid (Table 5).

3.2.4 Acid detergent fiber & neutral detergent fiber

Acid detergent fiber (cellulose and lignin) and neutral detergent fiber (hemicelluloses, cellulose, and lignin) are considered to be two important characteristics of forage quality (Caballero et al., 1995; Assafe & Ledin, 2001). High quality forages have low concentrations of both NDF and ADF. According to the results of variance analysis, irrigation treatments had a significant effect on ADF and NDF concentrations of forage. Both ADF and NDF follow an incremental trend as interval of irrigation increased (Table 3). Newman (2014) reported an association between the improvement of corn plant ADF and NDF content and drought stress. Similarly, Xu & Lascano (2007) reported that drought stress increased concentrations of both NDF and ADF forage by reducing the share of grain and ear in corn. Contradictory finding was reported by Küchenmeister et al. (2013) when they studied the effect of drought condition on ADF and NDF content of forage legumes; they found that drought stress reduced NDF and ADF concentration for forage legumes.

Further, in this study it was reported that ADF and NDF content was not affected by using zeolite (Table 2) but significant difference was reported in the value of ADF and NDF content in case of tested cultivars (P<0.05). Among various tested cultivars, lowest ADF and NDF content was reported from the KSC705 cultivar and KSC720 cultivars showed superiority over this (Table 6). Also, forage NDF content was influenced by the interaction of irrigation regime and hybrid (P<0.05). The highest NDF content (54%) was observed in combination of irrigation treatment Ir\(_{130}\) and KSC720 hybrid while the lowest NDF content was reported from the combination of treatment Ir\(_{100}\) and KSC705 hybrid (36.8%) (Table 5).

3.2.5 Dry matter digestibility

Improving the digestibility of forage is an important objective of breeding programs because high digestibility, improves forage intake and efficiency of conversion of nutrients by livestock. According, Coleman & Moore (2003) dry matter digestibility represents digestible energy. In this study, forage DMD significantly influenced by irrigation level and hybrid (Table 2). Opposite to ADF and NDF concentrations, forage DMD declined significantly (P < 0.01) as the interval of irrigation increased (Table 3). It could be due to increase of ADF concentration at higher interval of irrigation and negative correlation of DMD with ADF and NDF accumulation. Negative correlation between DMD and hemicelluloses has already been reported by various researcher in several similar studies (Theander & Westerlund, 1986; Hatfield, 1993; Contreras-Govea et al., 2009). Further, Wilson & Ng (1975) observed a better water status in maturing plants alleviated the extent of digestibility decrease in senescing leaves and stems. Study also suggested that application of Zeolite had no significant effect on DMD forage (Table 2). Further, hybrids had significant difference (P < 0.01) on DMD content (Table 2). The highest DMD concentration (69.5%) was recorded for KSC705 (Table 3). It would be due to the lower content of ADF in this hybrid compared with KSC704 and KSC720.

Conclusion

Results of study revealed a noticeable effect of water deficiency on the production of forage and all three selected cultivars had highest forage production at the Ir\(_{50}\) irrigation regime. Under such experimental conditions, irrigation levels seemed to be a more influential factor compared to zeolite with regards to most forage quality and quantity parameters. Zeolite increased forage and protein yield under different irrigation regimes but it did not show any significant effect on rest of the studied forage quality parameters. Therefore, considering the water shortage in the country and importance of silage corn as a forage plant, application of zeolite can be useful to save more water that leads to produce more yields. Overall, KSC704 hybrid showed superiority over the rest two hybrids.
due to greater forage dry matter production and higher protein yield. On the other hand, KSC705 produced more desirable forage in terms of some forage quality parameters.

Acknowledgements

The authors would like to thank respectable authorities on Seed and Plant Improvement Institute in Karaj, as well as Department of Agronomy, Faculty of Agriculture, Islamic Azad University of Karaj, Iran that helped us in carrying out this study.

Abbreviations

Ir<sub>70</sub> - Optimum irrigation Levels (at 70 mm evaporation)
Ir<sub>100</sub> - Moderate irrigation Levels (at 100 mm evaporation)
Ir<sub>130</sub> - Low irrigation Levels (at 130 mm evaporation)
CP - Crude protein
WSC - Water soluble carbohydrate
ADF - Acid detergent fiber
NDF - Neutral detergent fiber
DMD - Dry matter digestibility

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

Reference


Newman MA (2014) Defining the energy and nutrient content of corn grown in drought-stressed conditions and determining the relationship between energy content of corn and the response of growing pigs to xylanase supplementation. MSc thesis submitted to the Iowa State University, Pp 5-25.
Effect of different irrigation regimes and zeolite application on yield and quality of silage corn hybrids


EFFECT OF MULCHING ON SOIL NUTRIENT LOSS REDUCTION, CASE STUDY OF WESTERN LANDS KHUZESTAN PROVINCE, IRAN

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ABSTRACT

One major negative effects of wind erosion is the loss of fertile soil which reduce the soil productivity. This study was conducted to evaluate the effect of wind erosion on the loss soil fertility and organic matter. Further, the effect of mulching on the reduction of soil erosion was also studied in present study. Study was conducted at the West Khuzestan province of Iran and soil loss situation was created by using simulator. Results of study revealed that average soil wind erosion was 26.73, 24.58 and 16.36 Kg/m\textsuperscript{2}/h in Borvayeh, Alvan and Hoveyzeh, respectively. Significant difference was reported between the control (without use of any stabilizer) and samples treated with polymer. Average soil erosion is very high in soil without any stabilizer but the rate of erosion, compared to the control sample. The results of study showed that wind erosion causes loss of nitrogen in soil and it was reported maximum (0.008 Kg/m\textsuperscript{2}/h) in the soil of Hoveyzeh. Further maximum loss in phosphorus (1.1 Kg/m\textsuperscript{2}/h) and organic matter (0.49 Kg/m\textsuperscript{2}/h) was reported from the soil of Alvan. This nutrient loss may cause serious environmental and economic problems in the land exposed to long term erosion. The use of polymer and vegetable-based mulch with structural stability, on an average, reduced 99.5% loss soil elements (except in concentrations 15% vegetable-based mulch that reduced 13.2 % loss of soil elements). Use of polymers enhanced the stability and connections aggregates in surface soil through the formation of the surface layer and resistant to corrosive force winds prevent the dust, soil loss and nutrient loss.
1 Introduction

Soil is one of the main natural resources but from past few decades, this valuable natural resource is continuously destroyed and devalued by wind erosion (KohnehShahri & Sadeghi, 2005). Under arid and semi-arid climatic condition, this is the one of the most important environmental problems (Behera et al., 2007). Further, it also destroy agricultural land, cause expansion in desert areas, buried channels, contaminate the surface water, loss of plant tissues and reduce photosynthesis (National project management dust 2010). In recent years, this pollution of aerosols from dust storms as a result of wind erosion has become serious threatened for the health of citizens (Gravandi et al., 2016); recent studies suggested that 12% of cardiovascular and respiratory diseases in Kermanshah, Tabriz, Isfahan and Ahwaz, related to concentrations of more than 10-20μg/m³ dust particles (Ghozikali et al., 2015; Gravandi et al., 2016; Zalaghi, 2010).

More than 1.25 million hectares of the total area of the Kozestan province are the desert and in this sense it is the most vulnerable regions in the country. Currently in the Kozestan province there are more than 280 thousand hectares of critical resources and dust. In addition, more than 380 thousand preference dust, are becoming critical areas for dust production (Nourzadehhadad & Bahrami, 2016). With the spread of erosion in a region, topsoil is continuously removed. This effect is not seen often in real time but seriously, cause environmental and economic problems in the future and over time, to reduce the amount of soil nutrients, decreases soil productivity and rise need for fertilizers to maintain crop productivity (Bosede, 2010).

Anasiru et al. (2013) conducted a study in order to analyze the economic value of soil loss caused by soil erosion in Indonesia and reported that in studied four erosion units the rate of erosion is 406 tons per hectare per season and it causing the loss of 2648 kg of carbon per hectare per season, 230 kg of nitrogen per hectare per season, 30 kg phosphorus per hectare per season and 69 kg potash per hectare per season. Similar type of study was conducted by Li et al. (2007) in 2004 - 2006 at southern New Mexico land of America and reported that wind storms caused more than 25% loss in total organic carbon and nitrogen than 5 cm of soil. Colazo & Buschiazzo (2015) studied textural changes induced by wind erosion in cultivated soils of different granulometry for ass textural changes produced by wind erosion in Argentinean.

Result of this study showed reduction in clay accumulation in aggregates of larger sizes produced by agriculture, which indicates an increase in the risk of removal of these particles by wind in loamy soils (Colazo & Buschiazzo, 2015). By using 13C, technique Li et al. (2014) studied the effect of soil redistribution on soil organic carbon (SOC) and total nitrogen (TN) stocks in an agricultural catchment of northeast china and reported net losses in SOC and TN over the past 56 years and it was approximately 152 and 11 tons respectively. SOC and TN in the investigated catchment, erosion-induced SOC and TN losses per year are around 1·2×106 and 0·1×106 tons. Nourzadeh et al. (2013) studied the effects of soil moisture on the threshold friction velocity wind erosion, horizontal flux of sediment and dust concentration in Khuzestan province and reported that soil moisture increases the threshold friction velocity and has reduced sediment horizontal transport and dust concentrations.

Results of previous studies suggested that increasing the particle diameter at the soil surface and structural stability is the factors that can control surface abrasion and erosion. Application of polymer and soil stabilizer is considered as an agent of wind erosion control and soil conservation techniques in recent years. These polymers create a network on the soil surface that acted as a bridge between soil particles and by connecting the particles to each other and creating a more coarse aggregate soil which increases the aggregate stability (Abassi et al., 2010b). This study aimed to evaluate the effects of wind erosion on soil nutrient and soil organic matter loss in the land affected by wind erosion. Further, effect of polymer (polyvinyl acetate) and vegetable-based mulch (obtained from palm tree) on the reduction of soil nutrients loss was assessed by using open circuit simulators wind erosion, which was designed and built, in the area susceptible to wind erosion Khuzestan province.

2 Materials and Methods

In order to measure soil erosion, loss of nutrient and organic matter, soil samples were removed from the surface layer (up to 5 cm depth) of Alvan, Hoveyzeh and Borvayeh. The location of sampling sites has been shown in Fig (1). Soils of these selected areas are dry and have high salinity, sandy texture and arid rithermal regime with hyperthermic moisture regime.

Collected soil samples were taken in to the laboratory for further investigation. From these collected samples total nitrogen was estimated by Kjeldahl method (Bremner, 1982), phosphorus by Olsen method (Olsen & Sommers, 1982), potassium by extraction methods with ammonium acetate (Thomas, 1982) and organic materials were analyzed by Walkley & Black method (Walkley & Black IA, 1934).

Measuring the intensity of wind erosion and factors influencing this under natural conditions is always difficult and expensive (Lopez, 1998). Using of wind erosion simulators is one of the alternative methods (Mahmoud Abadi et al., 2011) and it can be quickly and effectively measured the soil properties as well as transport mechanisms under controlled conditions (Burri et al., 2011).

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Wind erosion simulators for this study was designed and built with five main components viz. (i) Fan tube wind generator with 2800 rpm, (ii) metal casing, (iii) a glass window for viewing soil particle transport mechanisms, (iv) digital Wind velocity meter, (v) inverter device to adjust the fan speed wind generator. Overview of developed device has been given in figure (4). After filling the trays from air-dried soil, mulch to a concentration from zero (control), 15%, 30% and 60% was sprayed on the soil surface so that cover the entire surface of the soil (Figure 2). The samples were placed in the natural environment for 72 hours, to mulch sprayed completely dry. After carefully weighing, tray (Scales with an accuracy of one gram), were placed in the desired location in device (Figure 3). In order to evaluate treatments against severe wind erosion, the maximum speed was considered the central axis of the tunnel. Maximum Speed the in central axis of the tunnel (1.18 inches above the soil surface) reached to 13 meters per second (47 Kilometer per hour). That correspond with maximum wind speeds of Khuzestan and more than wind erosion threshold is sampled areas.
The time for testing was considered 5 minutes. After completing the test, the samples are removed from the device and re-weighed. The difference in weight of samples at the beginning and end of the experiment was considered as the soil erosion.

In order to analyze the effect of polymer and vegetable-based mulch on the wind erosion, test results were analyzed in format of factorial experiment in a completely randomized design with 16 SPSS software.

### 3 Results and Discussion

Chemical properties of the studied areas soil samples, including texture, electrical conductivity and acidity have been indicated in Table 1. The average soil loss were evaluated at the speed of 13 meters per second under two types (polymer and vegetable-based mulch) in four levels of treatment 0 (control), 15%, 30% and 60% in simulators wind erosion devise in 5 minutes and the results of this these are presented in Table 2.

#### 3.1 The effect of mulches on soil erosion

Result of study revealed that the use of polymer and vegetable-based mulch (as a stabilizer) create a stable layer on the soil surface, which reduced the soil loss due to wind erosion. In terms of impact of stabilizer on the reduction of soil loss statistically no significant difference was reported between used two stabilizers but these two are significantly different than the control (Table 3).

Results shown in Table 2, suggested that average soil erosion, without any stabilizer are 26.73 kg/m²/h in Borvayeh sample, while it was reported 24.58 kg/m²/h in Alvan and 16.36 kg/m²/h in Hoveyzeh samples. Further, the rate of erosion in polymer treatments as compared to control decreased more than 99%. Result of study suggested that at 60% concentration of polymer, erosion decreased 0.61 kg/m²/h in the Borvayeh sample, while this value was 0.37 kg/m²/h and 0.61 kg/m²/h for Alvan and Hoveyzeh samples respectively.

### Table 1 The Physicochemical properties of the Alvan, Hoveyzeh and Borvayeh are soils.

<table>
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<tr>
<th>S. N</th>
<th>Soil properties</th>
<th>Sampling location</th>
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<td>10</td>
<td>Phosphorus (ppm)</td>
<td>0.1</td>
</tr>
<tr>
<td>11</td>
<td>Potassium (ppm)</td>
<td>125</td>
</tr>
</tbody>
</table>
Table 2 Average soil erosion in the use of polymer and vegetable-based mulch in speed of 13 m/s in wind erosion simulators for 5 minutes.

<table>
<thead>
<tr>
<th>Row</th>
<th>Soil</th>
<th>Concentration of mulch (%)</th>
<th>Polymer</th>
<th>Vegetable-Based Mulch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average erosion during the test (g)</td>
<td>Average erosion (kg/m²)</td>
</tr>
<tr>
<td>1</td>
<td>Borvayeh</td>
<td>0</td>
<td>219.2</td>
<td>26.73</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>15</td>
<td>10</td>
<td>1.22</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>30</td>
<td>8</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>60</td>
<td>5</td>
<td>0.61</td>
</tr>
<tr>
<td>5</td>
<td>Alvan</td>
<td>0</td>
<td>201.6</td>
<td>24.58</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>15</td>
<td>8</td>
<td>0.98</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>30</td>
<td>5</td>
<td>0.61</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>60</td>
<td>3</td>
<td>0.37</td>
</tr>
<tr>
<td>9</td>
<td>Hoveyzeh</td>
<td>0</td>
<td>134.6</td>
<td>16.36</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>15</td>
<td>15</td>
<td>1.83</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>30</td>
<td>5</td>
<td>0.61</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>60</td>
<td>5</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Results of statistical analysis that included two type of soil stabilizer (polymer and vegetable-based mulch), four levels (0, 15%, 30%, 60%), three soil types and three repeats have been shown in Table 3.

Table 3 Table analysis of variance effect of type mulch, concentration mulch and type soil on soil loss.

<table>
<thead>
<tr>
<th>Sources of changes</th>
<th>Degrees of freedom</th>
<th>Mean Square amount of soil loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type mulch</td>
<td>1</td>
<td>*3483920.056</td>
</tr>
<tr>
<td>concentration mulch</td>
<td>3</td>
<td>*4.369</td>
</tr>
<tr>
<td>type soil</td>
<td>2</td>
<td>*6908978.181</td>
</tr>
<tr>
<td>error</td>
<td>65</td>
<td>414087.329</td>
</tr>
<tr>
<td>total</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

ns : There is not a significant difference; ** There is a significant difference at 1%

According to Table 3, different concentrations of polymer are significant at 1%. Comparison of the average erosion by Duncan method revealed that there is no significant difference between mean wind erosion of soil at 30% and 60% concentrations of polymer with 15% and 30% concentrations, but there are significant differences between 15% and 60% mulch concentrations to reduce.

The average soil loss by wind erosion in Borvayeh sample treated with vegetable-based mulch decreased to 2.19 kg/m²/h at 60 concentration while this value was reported 23.25 kg/m²/h in case of 15% concentration. Similarly in case of polymer treatment, soil loss in wind erosion decreased to 0.61 kg/m²/h at 60 percent concentration while this was reported 1.22 kg/m²/h at 15 percent concentration.

Figure 5 Average soil erosion in Hoveyzeh samples treated with polymer and vegetable-based mulch.
These results are in agreement with the findings of Han et al. (2007) those who reported the influence of polymers on the reduction of soil erosion. These researchers evaluated soil erosion at 25.3 meters per second and reported 0-0.4 kg/m²/h erosion (Han et al., 2007). Further, the average soil erosion in the Borvayeh control soil was 26.73 kg/m²/h. The test results showed soil loss in 15, 30 and 60 percent concentrations of vegetable-based mulch was 23.22 kg/m²/h (13% reduction in erosion), 8.87 kg/m²/h (66.8% reduction in erosion) and 2.19 kg/m²/h (91.8% reduction in erosion) respectively, while soil loss in Borvayeh samples at treatments with polymer in 15, 30 and 60 percent concentrations was 1.22 kg/m²/h (99.5% reduction in erosion), 0.98 kg/m²/h (99.63% reduction in erosion) and 0.61 kg/m²/h (99.77% reduction in erosion) respectively. Khan (2014) investigated the effect of mulch (F2SR-231) on stabilizing the sand dunes and reported mulch resistance against winds (90 kilometers per hour), was confirmed in the wind tunnel. In deserts, positive effect of these polymers in reducing soil erosion and protecting plants and soils were reported by Khan (2014).
Polymer formed a perfectly smooth surface on the soil surface and this layer is free from any type of leaks and cracks, due to the high permeability in the soils it reached about 5 mm deep in to the sand and after drying it created the two layers. The top surface layer work as a solid sheet and has an integrated level and under this layer, attached soil particles are available without higher density.

Results of statistical analysis comparison between the types of soils showed that there is significant difference between wind erosion rate in the three soil samples. According to figure 8, the average wind erosion of Boryayeh, Alvan and Hoveyzeh samples at speed of 13 meters per second in simulators wind erosion was 26.73, 24.58 and 16.36 kilograms per square meter per hour, respectively. According the results of table 2, maximum soil loss in three sample sites at maximum speed (13 m/s) was reported from the samples collected from the Boryayeh localities and the soil of this locality have sandy texture with coarse grad and lacking organic matter. Duncan test showed that there is no significant difference between wind erosion rate at Alvan and Hoveyzezh sampling site but the soil samples collected from Boryayeh localities are significantly different from these two, which can be different because of the size distribution of soil particles, organic matter and the type of ions in soils. Nohegar et al. (2011) in a study the use of poly lattice polymer in Hormozgan province (Iran) and reported that used mulch had the ability to protect the sand against the wind (Nohegar et al., 2011).

### 3.2 The effect of mulches on nutrients loss in soils

Tables 4 and 5 showed the nutrients and organic matter losses in collected soil samples under wind erosion and impact of two types of mulch viz. polymer and vegetation-base mulch on the reduction of soil nutrients and organic matter loss reduction.

### Table 4 Effect of vegetable-based mulch on the reduction of soil nutrients loss.

<table>
<thead>
<tr>
<th>Type soil</th>
<th>Concentration of vegetable-based mulch (%)</th>
<th>Average erosion (kg/m²/h)</th>
<th>Nutrient loss (g/m²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nitrogen</td>
</tr>
<tr>
<td>Boryayeh</td>
<td>0</td>
<td>26.73</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>23.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8.87</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.19</td>
<td>0</td>
</tr>
<tr>
<td>Alvan</td>
<td>0</td>
<td>24.58</td>
<td>1.229</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.98</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.49</td>
<td>0.0245</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.61</td>
<td>0.0305</td>
</tr>
<tr>
<td>Hoveyzeh</td>
<td>0</td>
<td>16.36</td>
<td>0.818</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.73</td>
<td>0.0365</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.1</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.37</td>
<td>0.0185</td>
</tr>
</tbody>
</table>
Table 5 Effect of vegetable-based mulch on the reduction of soil nutrients loss.

<table>
<thead>
<tr>
<th>Type soil</th>
<th>Polymer concentration (%)</th>
<th>Average erosion (kg/m²/h)</th>
<th>Nutrient loss (g/m²/h)</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>Potassium</th>
<th>Organic matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borvayeh</td>
<td>0</td>
<td>26.73</td>
<td>0</td>
<td>2.67</td>
<td>38.22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.22</td>
<td>0</td>
<td>0.12</td>
<td>1.74</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.98</td>
<td>0</td>
<td>0.06</td>
<td>0.87</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.61</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Alvan</td>
<td>0</td>
<td>24.58</td>
<td>1.229</td>
<td>44.4</td>
<td>1.52</td>
<td>1.96</td>
<td>49.16</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.98</td>
<td>0.049</td>
<td>2.76</td>
<td>0.95</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.61</td>
<td>0.035</td>
<td>0.06</td>
<td>0.76</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.37</td>
<td>0.0185</td>
<td>1.68</td>
<td>0.57</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Hoveyzeh</td>
<td>0</td>
<td>16.365</td>
<td>0.818</td>
<td>1.637</td>
<td>20.45</td>
<td>32.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.83</td>
<td>0.0915</td>
<td>0.18</td>
<td>2.28</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.61</td>
<td>0.035</td>
<td>0.06</td>
<td>0.76</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.61</td>
<td>0.035</td>
<td>0.06</td>
<td>0.76</td>
<td>1.22</td>
<td></td>
</tr>
</tbody>
</table>

Results presented in table 4 and 5 revealed that in Borvayeh sample average soil loss was reported 26.73 kg/m²/h and due to wind total 2.67 g/m²/h phosphorus and 38.22 g/m²/h potassium reduction was reported. In this treatment, no reduction in the total nitrogen and organic loss was reported in this study. Further, average soil loss in Alvan sample was 24.58 kg/m²/h, while in case of nitrogen 1.229 g/m²/h, phosphorus 111.3 g/m²/h, potassium 38.34 g/m²/h and organic matter 49.16 g/m²/h reduction was reported due to wind erosion in simulators wind erosion device. On the other hand, in hoveyzeh sample soil loss was reported 16.37 kg/m²/h due to wind erosion in simulators. While due to wind erosion device 0.818 g/m²/h reduction in nitrogen, 1.637 g/m²/h in phosphorus, 20.45 g/m²/h in potassium and 32.73 g/m²/h in organic matter were reported. Ha-Lin et al. (2006) reported nature of the geological formations in arid and semi-arid conditions and reported that elements such as carbon, nitrogen and phosphorus concentrations are low under these condition, on the other hand, the land exposed to wind erosion gradually lost nutrients, dropped percentage of fine particles in them and rises PH.

As compare to other two localities, Alvan soil has higher amount of phosphorus that’s why maximum loss of phosphorus was also reported from the sample collected from the Alvan (111.3 g/m²/h) while it was reported 2.67g/m²/h and 1.637g/m²/h for the Borvayeh and Hoveyzeh sample respectively. Similarly, maximum loss of nitrogen was reported from the sample collected from Alvan (1.229 g/m²/h) while this value was reported 0.818g/m²/h for the sample collected from Hoveyzeh. Nitrogen concentration in Borvayeh sample is zero this may be due to the lack of organic matter, sandy texture and high loss of nitrogen in arid and semi arid areas (Abassi et al., 2010a). With due attention to higher rates of soil loss at Alvan sample due to wind erosion in the wind erosion simulators, the amount of organic material lost in this sample (49.16gr/m²/h) is higher than the Hoveyzeh sample (32.73 gr/m²/h).

Figure 9 Average loss of soil nitrogen in Alvan and Hoveyzeh soil samples in two mulch treatment
These results correspond with the finding of Lin et al. (2006) those who studied impact of wind erosion on the characteristics of sandy farmland in northern China. These researchers reported that wind erosion increased 6.2% sand particle, 3.7% pH, 2.2% temperature surface soil and decreased soil organic carbon, total nitrogen, total phosphorus, available nitrogen and soil moisture at rate 19.3%, 21.7%, 13.7% and 26.6% respectively (Lin et al 2006).

3.2.1 The effect of mulches on nitrogen loss in soils

Results of study suggested that use polymer in Alvan sample reduced the nitrogen loss from 1.229 to 0.049, 0.035 and 0.0185 g/m²/h on the concentrations of 15%, 30% and 60% respectively (table 4 & 5). Also similar type of reduction was reported in the Hoveyzeh sample 0.818 g/m²/h to 0.0915, 0.0305 and 0.0305 g/m²/h at the same concentrations of 15, 30 and 60% concentration respectively (Figure 9).

Compared to vegetable based mulching, polymer based mulching reduced nitrogen loss from 1.229 g/m²/h to 0.049, 0.0245 and 0.0305 g/m²/h at the concentrations of 15, 30 and 60% respectively. Similarly, use of polymer mulch also reduced nitrogen in Hoveyzeh sample from 0.818 g/m²/h to 0.0365, 0.055 and 0.0185 g/m²/h, respectively at various concentrations of 15, 30 and 60%. Wang et al. (2006) studied dust storms and erosion in the lands of northern China and reported that severe dust storms can reduce the amount of carbon and nitrogen in soil up to 66% and 73% compared to control treatment in eroded lands.

3.2.2 The effect of mulching on phosphorus loss in soils

Use of vegetable-base mulching in Borvayeh sample were reduced phosphorus, in control total phosphorus was reported 2.673 g/m²/h while in lowest dose treatment (15%) it was reported 2.32 g/m²/h. With increasing the level of vegetable based mulching, reduction in the phosphorus was reported and minimum phosphorus loss (0.217 g/m²/h) was reported from the treatment containing 60% mulch concentration. In case of polymer mulching, maximum 0.12 g/m²/h phosphorus losses was reported from the soil treated with 15% polymer concentration while the minimum phosphorus losses (0.06 g/m²/h) was reported from the soil treatment by 60% polymer concentration. Result of study revealed positive effects of polymer in reducing nutrient loss and it was superior to vegetable-base mulch (Figure 10).

Among the studied samples, Alvan sample treated with polymer had the highest phosphorus loss 111.3 g/m²/h in control while the maximum reduction in phosphorus loss 4.44 g/m²/h were recorded in the soil treated with 15% concentration of mulch and minimum 1.68 gr/m²/h in 60% concentration of polymer mulch.

<table>
<thead>
<tr>
<th>Mulch concentration</th>
<th>Alvan</th>
<th>Hoveyzeh</th>
<th>Borvayeh</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount of loss soil phosphorus (g/m²/hr)</td>
<td>15%</td>
<td>30%</td>
<td>60%</td>
</tr>
<tr>
<td>15%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 10 Compare the average loss of soil phosphorus in Alvan, Hoveyzeh and Borvayeh in treatment of two mulches.
3.2.3. The effect of mulches on organic matter loss in soils

Soil organic matter contented contains 94% Nitrogen and 25% to 50 % phosphorus. Majority of the organic matter was reported in topsoil as the result of erosion become out of reach, studies have shown that organic materials eroded by wind or water, is 1.3-5 degree higher than the soil remains in place (Ashna Abad & Ruhani, 2016). Organic matter in Borvayeh sample is zero which is one of the causes of higher average wind erosion and in effectiveness vegetable-base mulch (especially at low concentrations) was reported from this sample. Higher average wind erosion in Alvan sample caused during the test, more organic matter can be removed from Hoveyzeh sample.

According to figure 11, use of mulch in Alvan sample reduced organic matter loss 49.16 g/m²h in control to 0.74 g/m²h in 60% concentration polymer and 0.98 g/m²h in 30% concentration vegetable –base mulch. Also the loss of organic matter in Hoveyzeh samples treated with polymer were reduced organic matter loss of 32.73 g/m²h in control to minimum 1.22 g/m²h in 15% mulching while maximum reduction 0.74 g/m²h was reported in 60% concentration of vegetable –base mulch.

In general, adsorption of polymer to soil particles depends on polymer properties (molecular weight, type and charge density polymer), properties of the soil and its structure (soil type and texture, organic matter content and type of ions in soil). The nature of the interaction between the anionic polymer and the soil surface is not completely known, but hydrogen bonding and ligand exchange are two proposed mechanism for the interaction of these compounds with the soil (Lu et al., 2002).

Conclusion

In present study nutrient and soil organic matter loss and effect of two type mulch on reducing soil loss by wind erosion simulators were evaluated. Results of study revealed the effect of polymer and vegetable-base mulch on the reduction of nutrient loss. Mulching reduced 99% soil loss in the study area. But in Borvayeh soil mulching was not found effective in reducing soil loss at low concentrations. At 15% mulch concentration, wind erosion decreased only 13%, this can be due to differences in the distribution of soil particle size, amount and type of organic matter and type of ions in soils. Loss of topsoil by wind erosion will follow the reducing in nutrients of soil and in future this may cause serious environmental and economic problems for the country. Use of mulch examined, with structural stability, were reduced 99.5% average nutrient loss in soil of studied area (Except in 15% concentrations of vegetable mulch that nutrient loss was 13.2%).

Addition of polymer in soil creates linkage with soil particles and forms a long chain in the soil, which cause formation the interlocking between the various layers of the soil. This can reduces the rate of soil loss due to wind erosion. Stabilize soils disposed to wind erosion with mulch studied and planting native sapling and compatible with existing conditions are solution to resolving the problem of wind erosion and reduce dust phenomenon in the region.
Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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IPM APPROACH FOR THE MANAGEMENT OF WILT DISEASE CAUSED BY 
Fusarium oxysporum f. sp. lycopersici ON TOMATO (Lycopersicon esculentum)

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Ministry of Agriculture, Iraq

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ABSTRACT

This study was conducted to find out the effect of combined application of fluorescent Pseudomonas, spent mushroom compost and the fungicide (Carbendazim 50 % W.P) on Fusarium wilt disease infected tomato plants grown in solarized and non-solarized soil. Results of study revealed that inoculation of fluorescent Pseudomonas and spent mushroom compost have significant effect on the number and weight of tomato fruits per replicate with cost benefit ratio as compared to the control treatment having Fusarium oxysporum f. sp. lycopersici infection. No significance differences was reported among the various treatments imposed, and highest tomato fruit per plant (8.75 fruits/plant) was reported from the treatment containing only sterilized soil after 150 days of plantation this was followed by treatment containing P. fluorescens (7.35 fruits/plant), spent mushroom compost (7.00 fruits/plant), Carbendazim (7.00 fruits/plant) and spent mushroom compost with Pseudomonas fluorescens (6.90 tomato fruit/plant). Similar trends was reported in case of fruit weight and net return and treatment containing only sterilized soil show highest fruit weight (158.60g), maximum net return (113329 Rs/ha) and incremental cost benefit ratio (1:4.50). While minimum net return (0 Rs/ha) was observed in the treatment containing non sterilized soil and F. oxysporum infection.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.
1 Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important vegetable crop which widely grown by both small and large scale farmers; even it is common in kitchen and home gardens too. It's good source of Vitamins A, B and C and also ripens fruits is have antibiotic properties which is helpful in healing wounds (Baloch, 1994). Wilts disease of tomato is caused by *Fusarium oxysporum* f.sp. *lycopersici*. It is one of the highly destructive tomato disease which caused infection even in plant grown in greenhouse (Larkin & Fravel, 1998; Borrero et al., 2004). The pathogen enters through the plant roots and proliferates in the vascular tissues leading to breakdown of the water supply of the infected plants (Agrios, 2005). Typical symptoms of the disease are yellowing and wilting of leaves and it progressing upward from the base of the stem. Initially, only one side of a plant is affected but after some time these symptoms spread to the rest of the plant and finally kill the plant. Due to prolonged survival in soil as a saprophyte and as resistant structures, *F. oxysporum* is difficult to control (Khan & Khan, 2002; Borrero et al., 2004). Tomato yield loss due to *F. oxysporum* infection varies between 10 to 90% and it depends on the stage of the plant growth and the environmental conditions (Kumar & Sood, 2002; Singh, 2005).

Wilt disease of tomato can be easily control by the application of chemical fertilizers, but excess use of chemical fertilizers not only affect the quality of tomato fruit but also cause environmental pollution. Further, chemical fertilizers also caused severe damage to not target organisms. Now in these days, most of the researchers worked on the searching of alternative approach for the management of this disease. Various bio-control organisms help in reducing this pathogen infection. Among these *Pseudomonads fluorescens* is a non-pathogenic rhizobacteria which suppress the soil-borne pathogens through rhizosphere colonization, antibiosis and iron chelation by siderophore production (Elad & Chet, 1987; Lemanaceau et al., 1992; Pierson & Thomashow, 1992). Further, these bacteria have ability to promote plant growth, either by directly stimulating the plant or by suppressing pathogens (Ross et al., 2000; Haas & Defago, 2005; Carlier et al., 2008; Rovera et al., 2008; Rosas et al., 2009; Srinivasan et al., 2009). Various researchers have been tested the antagonistic properties of *F. oxysporum* (Madi et al., 1997; Tsahouridou & Thanassoulopoulos, 2002; Errakhi et al., 2007). Further researchers also established the fact that *F. pseudomona* can be used as a biological control agent against *F. oxysporum* (Elad, 1995; Singh et al., 2003).

Soil solarization is also a common practice for managing soil born diseases; it’s affect soil health, plant growth, crop yield, and quality of crop plants (Katan, 1987).

Like other management practices, soil solarization also used to control tomato wilt disease, (Ioannou et al., 2000; Tamietti & Valentino, 2006). Barakat & AL-Masri (2012) carried out soil solarization for the management of *F. oxysporum* f. sp. *lycopersici*, for seven weeks from July to August 2008 and 2009 and reported significant reduction in the population of the pathogen. Further, Raj & Kapoor, (1997) reported that mushroom compost also enhancing microbial activity in the amended soil and higher dosage (2%, w/w) of composts are most effective in managing the pathogen *F. oxysporum* f. sp. *lycopersici*, therefore it can be use for better plant health and disease control. It was well reported that mushroom compost (spent mushroom substrate, SMS, mushroom soil) exhibits suppressive characteristics against various fungi, as well as against plant diseases caused by fungi. In addition, mushroom compost has physical and chemical characteristics that make it ideal for blending with landscape mulch to enhance growth of horticultural plants (Davis et al., 2005). Incorporation of composted Spent Mushroom Substrate (SMS) not only improves the nutrient status but also neutralizes the acidity of soils (Pannier, 1993; Ahlawat et al., 2005) and facilitates cultivation even in problematic soils (Ahlawat et al., 2011). In addition, SMS also possesses good bio-control activity against certain foliar and soil borne diseases (Yohalem et al., 1996; Ahlawat et al., 2007).

Ajay & Shashi (2012) reported that effective control by 10 minute dipping of tomato seedlings roots in 0.3% solution of Carbendazim 50 WP before transplanting inhibited wilt disease caused by *F. oxysporum* f. sp. *lycopersici*. Amini (2009) evaluated carbendazim against *F. oxysporum* f. sp. *lycopersici in vivo*, the result of glasshouse tests revealed efficacy of fungicide in reducing disease infestation. The aim of this study was to evaluate the number of tomato fruits and cost benefit ratio of yield by using IPM approach with fluorescent *Pseudomonas*, soil solarization, spent mushroom compost and fungicide (Carbendazim).

2 Materials and Methods

This pot study was conducted during 2014 under net house condition at Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India. Experimental pots were laid out in Complete Randomized Block Design (CRBD) with six treatments and five replicates. Pot used in this study was of 10 cm in diameter and with capacity of 10 kg soil. Pots soil was artificially contaminant by adding pure culture of *F. oxysporum* f. sp. *lycopersici* @ 2 g/kg soil, this pure culture was multiplied on sorghum grains.

2.1 Process of soil solarization

Soil solarization was conducted for 2 months from 15th April to 15th June 2013 at research field of SHIATS, Allahabad. Soil was solarized with the help of 40 μm thick polythene sheet, soil was properly irrigated before laying the polythene sheet.

2.2 Source of Tomato seeds, fluorescent *Pseudomonas*, Spent mushroom compost and pathogen

Seeds of local tomato variety (CO-3) were collected from Indian Institute of Vegetable Research, Varanasi, Uttar...
Pradesh, India. Healthy seeds were selected manually and used for study. Fluorescent *Pseudomonas* was acquired from Yash Trichoguard, DBT Referral Lab, SHIATS, Allahabad, Uttar Pradesh, India while the culture of spent mushroom compost and *F. oxysporum* was acquired from Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Naini, Allahabad. Pure culture of *F. oxysporum* was maintained on czapek’s dox agar and mass culture of *F. oxysporum* was maintained on sorghum grains.

2.3 Application of fluorescent *Pseudomonas*, carbendazim and Spent mushroom compost

The solarized and unsolarized soil was mixed with FYM @ 100 g/pot and filled in the experimental pots. Tomato seeds were treated with bioagent *P. fluorescens* and chemical fertilizer Carbendazim @ 4g /kg seeds and shown in the pot @ 10 seeds per ponds. Simultaneously pot soil was inoculated with *P. fluorescens* and Carbendazim @ 2 g / pot. Ten pots were supplemented with spent mushroom compost @ 20 g / kg.

3 Results and Discussion

3.1 Effect of various treatments on fruit production

All studied combinations have statistically significant difference than the control (Non sterilized soil along with *F. oxysporum* inoculation). Among various tested treatments, treatment containing only sterilized soil without *F. oxysporum* shows superiority over the rest of the treatments and gave average 8.75 tomato fruit/plant after 150 days of plantation. This fruit number was followed by treatment containing solarized soil containing *P. fluorescens* (7.35 tomato fruit/plant), solarized soil along with spent mushroom compost (7.00 tomato fruit/plant), solarized soil and Carbendazim (7.00 tomato fruit/plant) and spent mushroom compost with *P. fluorescens* (6.90 tomato fruit/plant). These three treatments are not statistically different among themselves. Similar, type of findings was reported by Haruna et al. (2011) when they tried carbendazim for wilt disease management in tomato plant.

These researchers reported 12% improvement in fruit production rate on the application of carbendazim and compost. Average weight of five tomato fruits per replicate (g) was also significantly different and varies with the treatments. Like fruit numbers, treatment containing only solarized soil show superiority (158.60g) over all the other studied treatments. This fruit weight was followed by the combination of solarized soil and Carbendazim (132.50g), solarized soil along with spent mushroom compost (131.60g), solarized soil along with *P. fluorescens* (116.20g) and spent mushroom compost with *P. fluorescens* (97.20g). These treatments are significantly different that the control (Non Solarized soil + *F. oxysporum*) but are not significantly different when compare with each other except compost with *P. fluorescens*. These results are in agreement in the findings of Seleim et al. (2011) those have reported highest increases in tomato yield by the application of *P. fluorescens*.

3.2 Cost benefit ratio

Data with respect to agronomical practices were same for all treatments (Table 2) while the economic values of all treatments were significantly different between treatments (Table 3). Like fruit characteristics, maximum net return (113329 Rs/ha) was recorded from the treatment containing solarized soil, this cost benefit ration was followed by treatment containing solarized soil with *P. fluorescens* (72871 Rs/ha), Carbendazim (67819 Rs/ha), Spent mushroom compost, (58329 Rs/ha) and Spent mushroom compost with *P. fluorescens* (40617 Rs/ha). The minimum net return (0 Rs/ha) was observed from the treatment containing Non solarized soil along with *F. oxysporum*. These researchers reported 12% improvement in fruit production rate on the application of carbendazim and compost. Average weight of five tomato fruits per replicate (g) was also significantly different and varies with the treatments. Like fruit numbers, treatment containing only solarized soil show superiority (158.60g) over all the other studied treatments. This fruit weight was followed by the combination of solarized soil and Carbendazim (132.50g), solarized soil along with spent mushroom compost (131.60g), solarized soil along with *P. fluorescens* (116.20g) and spent mushroom compost with *P. fluorescens* (97.20g). These treatments are significantly different that the control (Non Solarized soil + *F. oxysporum*) but are not significantly different when compare with each other except compost with *P. fluorescens*. These results are in agreement in the findings of Seleim et al. (2011) those have reported highest increases in tomato yield by the application of *P. fluorescens*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average number of fruits / plant</th>
<th>Average weight of five fruits / replicate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 90</td>
<td>120</td>
</tr>
<tr>
<td>Non SS along with Fo</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>SS along with Smc and Fo</td>
<td>1.70</td>
<td>5.85</td>
</tr>
<tr>
<td>SS along with Pf and Fo</td>
<td>0.80</td>
<td>6.15</td>
</tr>
<tr>
<td>SS in combination with Smc + Pf + Fo</td>
<td>0.25</td>
<td>5.80</td>
</tr>
<tr>
<td>SS along with C +Fo</td>
<td>3.25</td>
<td>5.75</td>
</tr>
<tr>
<td>SS along with tomato plant</td>
<td>1.20</td>
<td>4.75</td>
</tr>
<tr>
<td>C. D. (P = 0.05)</td>
<td>1.765</td>
<td>1.974</td>
</tr>
</tbody>
</table>

Here SS - Solarized soil; Fo – *F. oxysporum*; Smc - Spent mushroom compost; C- Carbendazim and Pf- *P. fluorescens*
Table 2 Estimated Cost of production based on the amount spend on agronomical practices for cultivation/ha (Pot data is converted in to field data).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Particular</th>
<th>Requirement</th>
<th>Rate/unit Rs.</th>
<th>Cost (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(A)</td>
</tr>
<tr>
<td>I.</td>
<td>Ploughing</td>
<td>3 hours</td>
<td>500 Rs/hours</td>
<td>1500</td>
</tr>
<tr>
<td>II.</td>
<td>Harrow</td>
<td>3 hours</td>
<td>500 Rs/hours</td>
<td>1500</td>
</tr>
<tr>
<td>III.</td>
<td>Layout of field</td>
<td>10 labours</td>
<td>150 Rs/labour</td>
<td>1500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(B)</td>
</tr>
<tr>
<td>I.</td>
<td>FYM</td>
<td>20 tons</td>
<td>100 Rs/qu.</td>
<td>20000</td>
</tr>
<tr>
<td>II.</td>
<td>Urea</td>
<td>193 Kg</td>
<td>7 Rs/Kg</td>
<td>1351</td>
</tr>
<tr>
<td>III.</td>
<td>DAP</td>
<td>174 Kg</td>
<td>15 Rs/Kg</td>
<td>2610</td>
</tr>
<tr>
<td>IV.</td>
<td>Labour</td>
<td>6 labours</td>
<td>150</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(C)</td>
</tr>
<tr>
<td>I.</td>
<td>Seed material</td>
<td>0.5 kg</td>
<td>1500 Rs/Kg</td>
<td>750</td>
</tr>
<tr>
<td>II.</td>
<td>transplanting and leveling</td>
<td>12 labours</td>
<td>150</td>
<td>1800</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(D)</td>
</tr>
<tr>
<td></td>
<td>Weed Management</td>
<td>15 labour X3 time</td>
<td>150 Rs/labour</td>
<td>6750</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(E)</td>
</tr>
<tr>
<td></td>
<td>Harvesting</td>
<td>30 labours</td>
<td>150 Rs/labour</td>
<td>4500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(F)</td>
</tr>
<tr>
<td></td>
<td>Total cost of cultivation</td>
<td></td>
<td></td>
<td>25161</td>
</tr>
</tbody>
</table>

Table 3 Estimated cost of various treatments formulation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cost of Smc + Pf + carbendazim (Rs)</th>
<th>Labor cost (Rs )</th>
<th>Total Cost (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non SS along with Fo</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SS along with Smc and Fo</td>
<td>20000</td>
<td>900</td>
<td>20900</td>
</tr>
<tr>
<td>SS along with Pf and Fo</td>
<td>200</td>
<td>900</td>
<td>1100</td>
</tr>
<tr>
<td>SS in combination with Smc + Pf + Fo</td>
<td>20200</td>
<td>900</td>
<td>21100</td>
</tr>
<tr>
<td>SS along with C +Fo</td>
<td>280</td>
<td>900</td>
<td>1180</td>
</tr>
<tr>
<td>SS along with tomato plant</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Here SS - Solarized soil; Fo – F. oxysporum; Smc - Spent mushroom compost; C- Carbendazim and Pf- P. fluorescens

Similarly, maximum cost benefit ratio and incremental cost benefit ratio were obtained with treatment containing solarized soil (1:4.50), this was followed by the treatment containing solarized soil along along with P. fluorescens (1:2.77), Carbendazim (1:2.57), Spent mushroom compost (1:1.26), and Spent mashroom compost with P. fluorescens (1:0.87). The minimum cost (0) benefit ratio and incremental cost benefit ratio was reported from Non solarized soil with F. oxysporum (Table 4).

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

References


Table 4 Impact of various treatments on the Cost benefit ratio of tomato.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>M</th>
<th>N</th>
<th>A</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>B</th>
<th>H</th>
<th>O</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non SS along with Fo</td>
<td>0</td>
<td>25161</td>
<td>25161</td>
<td>0</td>
<td>0</td>
<td>22000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SS along with Smc and Fo</td>
<td>20900</td>
<td>25161</td>
<td>46061</td>
<td>4.745</td>
<td>4.745</td>
<td>22000</td>
<td>104390</td>
<td>104390</td>
<td>58329</td>
<td>1:1.26</td>
<td>58329:1:1.26</td>
</tr>
<tr>
<td>SS along with Pf and Fo</td>
<td>1100</td>
<td>25161</td>
<td>26261</td>
<td>4.506</td>
<td>4.506</td>
<td>22000</td>
<td>99132</td>
<td>99132</td>
<td>72871</td>
<td>1:2.77</td>
<td>72871:1:2.77</td>
</tr>
<tr>
<td>SS in combination with Smc + Pf + Fo</td>
<td>21100</td>
<td>25161</td>
<td>46261</td>
<td>3.949</td>
<td>3.949</td>
<td>22000</td>
<td>86878</td>
<td>86878</td>
<td>40617</td>
<td>1:0.87</td>
<td>40617:1:0.87</td>
</tr>
<tr>
<td>SS along with C +Fo</td>
<td>1180</td>
<td>25161</td>
<td>26341</td>
<td>4.280</td>
<td>4.280</td>
<td>22000</td>
<td>94160</td>
<td>94160</td>
<td>67819</td>
<td>1:2.57</td>
<td>67819:1:2.57</td>
</tr>
<tr>
<td>SS along with tomato plant</td>
<td>0</td>
<td>25161</td>
<td>25161</td>
<td>6.295</td>
<td>6.295</td>
<td>22000</td>
<td>138490</td>
<td>138490</td>
<td>113329</td>
<td>1:4.50</td>
<td>113329:1:4.50</td>
</tr>
</tbody>
</table>

Here SS - Solarized soil; Fo – F. oxysporum; Smc - Spent mushroom compost; C- Carbendazim and Pf- P. fluorencens.


Amini J (2009) Physiological race of Fusarium oxysporum f.sp. lycopersici in Kurdistan province of Iran and reaction of some tomato cultivars to race 1 of pathogen. Plant Pathology Journal 8: 68–73. DOI: 10.3923/ppj.2009.68.73.


ABSTRACT

This study was aimed to survey the prevalence of Salmonella spp. in water sources of Sistan and Baluchistan Province, Iran. Total 100 samples were collected from different sites and divided on the basis of potability, geographic location, accessibility, consumption and water flow types. Protocols issued by Institute of Standards and Industrial Research of Iran were used to detect Salmonella spp. Results of study revealed that 74.6% water sources shows the presence of Salmonella spp. Among studied sources, only one sample of potable water was contaminated with Salmonella spp. The highest contamination was reported from still water and it was significantly different that the pipe water (p<0.05). The highest contamination among non-potable water was reported from Jazinak compared to other regions. Further, it was reported that non-potable water is mostly used for non-agricultural consumption and it was found to be more polluted than water used for irrigation (p<0.05). The highest contamination with Salmonella was reported in ponds water (p<0.05). Non-potable water collected from east region of Sistan was reported more polluted than the west region. Contamination of non-potable water resources in the study area was high. Potable water, totally, indicates the proper function of the Salmonella treatment plan of water refinery of Sistan. Non-potable water of study area is polluted. Identification of Salmonella serotype and antibiotic susceptibility testing serve as indicators to define the accurate level of contamination.
1 Introduction

Contaminated water plays an important role in diseases transmission and it performs a vital role in the public health and survival of organisms, especially in rural areas of developing countries (Makoni et al., 2004; Pant, 2004; Katsi et al., 2007). People from developing countries encountered with higher risk of water-borne diseases as compared to the developed countries (Simpson et al., 2002). Based on the report of World Health Organization (WHO), the mortality rate of diseases related to contaminated water is more than 5 million people per year. Nowadays, efforts to attain higher quality of drinking water were emphasized (WHO, 2008).

Microorganisms are of notable importance in many aspects of water quality control and drinking water could transfer disease-causing bacteria, viruses and parasites (Tebbutt, 1977; Cabral, 2010). Generally, drinking water contains two categories of microorganisms first is known as persistent microorganisms which naturally settle in water, with little food needs, and it includes Acinetobacter, Flavobacterium and Chromobacterium species while other group is transient microorganisms which are transmitted to water from the environment, soil, human or animal, and pathogens fall into this category (Barati, 2011). On global scale, drinking water pollution by pathogens is evaluated the most important risk for human health, and also it has been lead to numerous outbreaks of diseases and poisoning (WHO, 2008). Microorganisms that cause disease via ingestion of contaminated water includes species such as Salmonella, Shigella, Escherichia coli, Vibrio cholerae, Campylobacter jejuni, Cryptosporidium, Entamoeba histolytica, Giardia and Balantidium coli (Cabral, 2010).

Enterobacteriaceae is one of the most important families among bacteria, is of great importance in medicine, industry and research areas and Salmonella spp. is a member of this family. It is gram-negative, motile and considered as the most important human and animal pathogen (Ramírez-Castroillo et al., 2015). Recent studies showed that Salmonella has more than 2,500 serovar and among these S. enteritidis and S. typhimurium are the most important one and the most isolated serotypes from water (Tabatabayi & Firouzi, 2002; Popoff & Le Minor, 2005; Tortora, 2008; Salem & Metawe, 2013). Eating contaminated food or drinking contaminated water is one of the major routes of Salmonella transmission (D’Aoust, 1989; Quinn et al., 2002; Brooks et al., 2015). Salmonella infection in humans is mainly caused by drinking water contaminated with secretions and feces of infected animals (Zahraei Salehi, 2000; Quinn et al., 2002; Winn et al., 2006; Motlagh et al., 2013).

One of the most common diseases caused by Salmonella in humans is typhoid fever which mostly spreads through contaminated food and water. Symptoms of typhoid fever in humans include fever, headache, abdominal pain, nausea, vomiting, diarrhea, gastroenteritis and septicemia (Acha & Szyfres, 2003; Bergeron et al., 2011; Chandra et al., 2013). According to Seas et al. (2000) children under five years-age, mainly in Asian and African countries are considered to be the most significant group of patients infected with waterborne microbial disease. Sources of drinking water, in many rural areas, are severely associated with the failure of quality control (Guppy & Shantz, 2011), and at the best form, it is only carried out once when water plans startup (Rossiter et al., 2010). Increased water scarcity on the one hand and low quality on the other hand, especially in many developing countries, are serious problems and have derived from misuse and mismanagement of water resources in these areas (Kakonge, 2002; Saravanan et al., 2011). Since no research has been conducted regarding the prevalence of one of the most important pathogens transmitted through water in Sistan city, the present study was performed to determine the prevalence of Salmonella spp. in water sources of Sistan region, Sistan and Baluchestan province, Iran, using conventional methods.

2 Materials & Methods

2.1 Sample Collection and Classification

This is a descriptive, cross-sectional study in order to determine the prevalence of Salmonella in water sources of Sistan. Study was conducted for 5 months (March 2014, to July 2015), water samples were collected from different water sources and areas of Sistan, including three main political division (Figure 1). Zabol is a city in Sistan and Baluchestan Province, Iran, and lies on the border with Afghanistan. Zabol is located near Lake Hamun and the region is irrigated by the Harmand River. Lake Hamun is a seasonal lake that is often dry and included three parts (Figure 1). Zabol is connected by road to Zaranj across the border in Afghanistan (Karimi et al., 2013). Sterile glass bottles with a volume of 300 ml were used for sampling. Tape water samples were collected as follow: for a minute, the faucet was left open and then water was gathered. In the cases where there was no direct access to water (shallow wells, agricultural canals, rivers and etc.) a rope was used: the bottles were tied up tightly, and then, completely immersed in water. As far as possible, by dipping the bottles, efforts were made to collect water from deeper parts of sites in order to prevent of exposure to air and surface contamination (about 30 cm).

In all cases, after obtaining the sample, the top of the bottles was sealed by parafilm and covered by an aluminum paper. The bottles were tagged, coded and transferred to Laboratory of Microbiology, Faculty of Veterinary Medicine, University of Zabol, Iran. Due to the distance and environment temperature, if necessary, the samples were delivered in the presence of ice. In total, 100 samples were collected randomly with an average of three replications. A total of 80 samples, based on the way of water flow were divided into three categories: still water (n=31), running water (n=19) and pipeline water (n=30).

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http://www.jebas.org
Also, 80 samples, based on the drinkability, were divided into two groups: potable (n=30) and non-potable water (n=50). Non-potable water was divided into three categories based on the state divisions: Sheyb-e-Ab (n=11), Posht-e-Ab (n=9), and Jazinak (n=28), into two categories based on consumption: used for irrigation (n=17) and non-irrigation (n=33) purposes, into five categories on the basis of water access: channel (n=10), river (n=7), pond (n=27), well (n=2) and dam (n=4) water, and into two categories based on the topography of Sistan: waters collected from East (n=28) and West region of Sistan (n=20). 20 samples of potable water that had been taken from different districts of Sistan, based on the geographical location were divided into two categories: eastern (n=8) and western (n=12) regions of the city.

2.2 Investigation the presence of Salmonella

Protocol No. 8789 issued by Institute of Standards and Industrial Research of Iran was used to detect the presence of Salmonella spp. (ISIRI, 2009). Briefly, three phases including pre-enrichment, enrichment and cultures in special and differential media were used.
In the pre-enrichment stage, 25 ml of water sample was added to 225 ml of Lactose Broth (Merck, U.S.A) and was incubated for 48 hours at 37 °C. Then, as enrichment phase, 1 ml of cultured Lactose Broth was added to the tube containing 10 ml of Tetrathionate broth (Merck, U.S.A) and also, 1 ml of cultured Lactose Broth was added to the tube containing 10 ml of selenite cystine broth (Himedia, India). These tubes were incubated for 24 hours at 37 °C.

In the third stage, a loopful of the tetrathionate and selenite cystine broths were inoculated on Salmonella Shigella Agar (SSA) plates (Merck, USA). The cultured media were incubated for 24 hours at 37 °C. Suspected colonies grown on SSA were subcultured on TSI agar (Liofilchem, Italy), SIM agar (Merck, USA), Simon citrate (Liofilchem, Italy), urea broth (Merck, USA), Methyl Red broth (Himedia, India), and Voges–Proskauer broth (Himedia, India) to confirm the presence of Salmonella. Salmonella organisms appeared as transparent or translucent colorless colonies with black dot on SSA. An alkaline/acid (red slant/yellow butt) reaction was indicative of Salmonella with a blackening of the medium. On SIM, A positive H2S test was denoted by a blackening of the medium along the line of inoculation and a positive motility test was indicated by a diffuse zone of growth flaring from the line of inoculation. A yellow color denoted a negative indole test after addition of Kovacs Reagent by Salmonella. A positive reaction was indicated by growth with development of a deep blue color reaction within the medium. Methyl red and Voges–Proskauer tests were positive and negative for Salmonella, respectively. The solution remaining yellow for methyl red indicated a negative test. After adding both alph-anaphthol and potassium hydroxide as reagents to Voges–Proskauer test, the tube was shaken vigorously, and then allowed to sit for 5-10 minutes. A pinkish-red color indicates a positive test (Quinn et al., 2002).

2.3 Analysis of Data

Data were analyzed with SPSS and K-square test. Significance value was considered to be less than 0.05.

3 Results

Result of study revealed that 74.6% water sources of the Sistan area have Salmonella contamination (Table 1). According to the results, types and status of water samples affect the Salmonella contamination in study area. Among the studied samples highest Salmonella contamination (31.2%) was reported from still water, it was followed by the running water (15%) and tap (pipeline) water (1.2%). K-square test showed a significant relationship between the water flow and the Salmonella contamination in such a way that the still water had the highest contamination and pipe water had the lowest contamination (p <0.05). The Salmonella contamination was observed 46.2% and 1.2%, in non-potable and potable water, respectively. K-square test showed that the Salmonella contamination is significantly higher in non-potable water compared to potable water (p <0.05). The results demonstrated that the Salmonella contamination in Jazinak, Posht-e-Ab and Sheyb-e-Ab regions are 37.5%, 18.7% and 16.7%, respectively; although the highest contamination was observed in Jazinak but this difference was not significant (p> 0.05).

Based on the results, Salmonella contamination in non-potable water used for irrigation and non-irrigation consumption was reported 12% and 62%, respectively. With a significant difference, the water used for non-irrigation purposes is significantly higher contaminated compared to water used for irrigation purposes (p<0.05). The results showed that Salmonella pollution was 50% in ponds, 12% in rivers, 6% in channels and 6% in dams. Further, it was reported that well water have less or almost nil contamination. K-square test showed that this difference was significant (p<0.05). Salmonella contamination of non-potable water in East and West regions of Sistan was 36% and 34%, respectively and based on K-square test, there was no significant difference (p> 0.05) was reported among various treatments. Among potable water samples, only one (5 %) was Salmonella contaminated, which was located in the West of the city and K-square test showed a statistically significant difference (p < 0.05). The rest of potable water samples collected from different areas of the city were negative for Salmonella.

4 Discussions

Today, there is no cost-effective substitute instead of water. As a result, water conservation and re-use would be wisely (Mercer, 1964; EL-Jakee et al., 2009). The water which has no negative effect on growth, reproduction and productivity of livestock and poultry is considered as healthy water and this is the fundamental basis for the growing healthy animal (Tebbutt, 1977; Duguma et al., 2012). At global scale, contamination of potable water by pathogenic bacteria leads to the main hazards to human health. Countless outbreaks of water-borne diseases are the result of the exposure of organisms to untreated or poorly treated water (Faruque et al., 1998; WHO, 2008; Salem & Metaewe, 2013). To best of our knowledge, this study is the first report which assesses the prevalence of Salmonella spp., as a zoonosis and water born pathogen, from the border region water sources of the Sistan, Iran. Salmonella spp. is considered as a most common cause of food-borne infection throughout the world (D’Aoust, 1989; Baird-Parker, 1990; Waage et al., 1999). Although the concentration of Salmonella in the water has been measured low but the consumption of contaminated water with this bacterium will be lead to infection, because the water can quickly pass through the stomach and then, enter to the intestines, without triggering the mechanisms of digestion and thereby the microorganism escapes the natural host defense systems. In addition, Salmonella can survive for a months in water (D’Aoust, 1989; Murray, 1991; Waage et al., 1999; Salem et al., 2011).
The contamination of water with *Salmonella* spp. in the study area was observed 74.6%, as a first report. Few previous studies related to the *Salmonella* contamination in the water sources of various regions of the Iran was investigated by the various researchers. In this context, Montaz et al. (2013) examined the presence of *Salmonella* spp. in packaged potable water and tap (pipeline) water in Iran. These researchers investigated the quality and contamination of potable water in Isfahan by PCR. Their results showed that 2.08% of the samples were contaminated with *Salmonella*. Further, Jafari et al. (2006) tested the pollution of drinking water with *Salmonella* in 40 broiler farms located in the rural areas of Ahvaz, Iran and reported the presence of *Salmonella* from five broiler farms.

The presence of *Salmonella* spp. in water sources can be a serious danger for the human and an animal community. Because of high contamination of water sources with *Salmonella* spp. in the Sistan region, further examination is required in order to achieve the origin of contamination and purgation of the sources. In present study, variation in the percentage of water contamination with *Salmonella* was reported from the still water and it was followed by running water, and pipes water that have had the least contamination. Still water has higher contamination than the running water and the K-square test revealed the significant difference.

It was found that there was a significant relationship between the water flow and the amount of *Salmonella* contamination. Indeed, the surface water that has movement, have less contamination than still surface water. Studies showed that water pollution was higher among those who stored water and used it later on. In fact, storage of water beside the immobility, especially in storage devices, in addition to the animal feces and also birds that loaf freely in home, even rodents and arthropodes like flies, as a mechanical carrier, can be act as factors to increase the pollution of water. Further investigations are required for the correct estimation of *Salmonella* population (Osman et al., 2010; Salem & Metawe, 2013).

In present study, *Salmonella* contamination in non-potable water was significantly higher than potable water and as mentioned earlier, this represents the proper function plan designed for water treatment of Sistan for *Salmonella*. The reported contamination also needs further inquiry to determine the predominant serotypes of this bacterium and their association with human diseases to increase the quality of water sanitation, effectively. In this study, analysis showed no significant difference among non-potable water of Sheyb-e-Ab, Posht-e-Ab and Jazinak regions, albeit, the descriptive results show higher contamination of water collected from Jazinak, followed by Posht-e-Ab. This issue might be related to existence of more water resources in Jazinak region for agriculture and livestock consumption. In this study, descriptive results showed *Salmonella* contamination of non-potable water used for non-irrigation purposes is higher compared to non-potable water used for irrigation purposes; and analytical results showed a statistically significant difference.

Major sources of surface contaminants include animal feces and other animal activities, leakage of contaminated material from shallow lakes or ponds, and landfill locations where debris and waste accumulate without sanitary mechanisms; on the other hand, groundwater contaminants sources consist of, for example, the contaminated tank with systems malfunction, underground tanks for domestic liquid sewage (sinkholes) or leaking underground sewer lines (Berger, 2012; Salem & Metawe, 2013). Each of the above-mentioned factors can lead to contamination in the region, however, further researches should be carried out in this regard. The descriptive results, regarding non-potable water, showed that the ponds were the most polluted, followed by rivers, channels and dams with similar degree of contamination, and ultimately wells with least contamination. K-square analysis showed, significantly,

<table>
<thead>
<tr>
<th>Flow type</th>
<th>Potability</th>
<th>Non-potable water</th>
<th>Drinkable water</th>
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<tbody>
<tr>
<td>still water</td>
<td>31.2</td>
<td>46.2</td>
<td>34</td>
</tr>
<tr>
<td>running water</td>
<td>15</td>
<td>1.2</td>
<td>34</td>
</tr>
<tr>
<td>tap (pipeline)</td>
<td>1.2</td>
<td>18.7</td>
<td>36</td>
</tr>
<tr>
<td>non-potable water</td>
<td>16.7</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>potable water</td>
<td>12</td>
<td>62</td>
<td>5</td>
</tr>
<tr>
<td>irrigation consumption</td>
<td>50</td>
<td>12</td>
<td>95</td>
</tr>
<tr>
<td>non-irrigation consumption</td>
<td>50</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>ponds water</td>
<td>12</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>rivers water</td>
<td>12</td>
<td>6</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>channels water</td>
<td>6</td>
<td>6</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>dams water</td>
<td>36</td>
<td>34</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>East region of study area</td>
<td>95</td>
<td>5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>West region of Sistan</td>
<td>62</td>
<td>36</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>West region of Sistan</td>
<td>36</td>
<td>34</td>
<td>p&lt;0.05</td>
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Table 1 prevalence (%) of *Salmonella* in various samples of study area.
the relationship between the way of access to non-potable water and contamination with *Salmonella*. Role of environmental pollutants of surface water in the study area could explain the cause of this result. Due to water rinsing, through seasonal raining, especially in confronting with environmental contaminant and feces of animal in different areas, the amount of microbial contamination would be increased in ponds (Mahmodi & Javanmardi, 2009).

The descriptive results regarding non-potable water contamination with *Salmonella* showed that contamination in East area of Sistan was higher than West area but analytical results did not detect significant differences. *Salmonella* detection in water may be conflict by low number and sometimes their intermittent presence (D’Aoust, 1989; Waage et al., 1999). In the current study, only one sample of drinking water was *Salmonella*-contaminated and the rest of potable water was negative for *Salmonella*. Studies showed that water contamination with *Salmonella* can be due to biofilm formation of opportunistic bacteria in the water distribution system, especially in surface of damaged pipelines, and release the bacterium in water (Edberg, 1996; Adesoji & Ogunjobi, 2013). Further investigation must be carried out to find out the origin of reported contamination. Our results may be due to increased resistance of bacteria to the high concentration of chlorine or other disinfectants used in the study area, albeit further studies are required to clarify this issue (Adesoji & Ogunjobi, 2013).

In conclusion, present study indicated that the water sources of Sistan region were severely contaminated with *Salmonella* spp. and identification of different serotypes of *Salmonella* in the study area would be worthwhile. On the other hand, the efficiency of water treatment system in Sistan, according to the results of this study, was reported well for *Salmonella* spp. Our study contributes in the assessment of water quality in Sistan, Iran. Although other water quality indicators need to include, but alarming signal for human consumption and public health was observed. Management of wind, *Salmonella* serotype identification and finally antibiotic susceptibility testing could be suggested to find out the risk factors of pollution.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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Prevalence of Salmonella spp. in water sources of Sistan: a descriptive cross-sectional study.

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KEYWORDS
Shoulder
Synovial chondromatosis
Open arthrotomy

ABSTRACT

Synovial chondromatosis is the rare and benign metaplasia of the synovial membrane resulting in the formation of multiple intra-articular cartilaginous bodies. This study was conducted on 28 years old male who was suffering from severe pain and limitations of motion in right shoulder since 2 years ago, which developed gradually, accompanied with night pain. Previous results and available literature suggested the use of arthrotomy or arthroscopy for the treatment of synovial chondromatosis of shoulder. In present study also both the treatment methods are effective but lesion open arthrotomies are found more effective due to some cases related to extension. Further, it was concluded that better results can be obtained on the early intervention of disease.
1 Introduction

Leannac (1813) was the first one who described the term synovial chondromatosis while the current knowledge regarding this disease was based on the description given by Jaffe in 1958 (Jaffe, 1958; Crotty et al., 1996; Dorfman & Czerniak, 1998; Fanburg-Smith, 2003; Hopyan et al., 2005). Recently used, cytogenetic evaluation suggested that synovial chondromatosis is a rare benign neoplasm which is caused by metaplasia of the synovium into chondrocytes (Springer, 1991; Mertens et al., 1996). Synovial chondromatosis is characterized by the formation of metaplastic and multiple foci of cartilage in the intimal layer of the synovial membrane of a joint (Davis et al., 1998a; Tachdjian, 1990). The aetiology of synovial chondromatosis is indecisive but Miligram (1977) classified the three stages viz early (active intrasynovial disease but no loose bodies), transitional disease (active disease and loose bodies), and late (multiple loose bodies but no intrasynovial disease).

The term synovial osteo-chondromatosis is used when the cartilage is ossified. The lesion also occurs in bursae and tendon sheaths (Sviland & Malcolm, 1995). This condition usually seen in knee, hip, or elbow, but it is rare in the shoulder (Varma & Ramakrishna, 1976, Volpin et al., 1980, Leo & Nocera, 1981). It usually occurs in persons older than 40 years of age but occasionally occurs in adolescents It predominantly occurred in men and frequency of synovial chondromatosis in men is twice than the women.

Arthrotomy caused the thickened synovium and studded with innumerable small, firm, flat or slightly raised grayish-white nodules. These cartilaginous or osteocartilaginous foci may become pedunculated and detached from the affected membrane, entering the joint cavity as loose bodies. Histologic studies disclose numerous foci of cartilaginous metaplasia of the synovium, which may be calcified or ossified.

Clinical complaint consist pain, swelling and stiffness to the affected joint. Further, joint may lock when there is loose body (Murphy et al., 1962; Trias & Quintana, 1976; Roulot & Le Viet, 1999; Butt et al., 2005). Month or years may elapse before patients seek treatment. On examination the synovial membrane is noted to be thickened and the joint is limited in its range of motion. Other physical signs that can be elicited are crepitus and palpable loose bodies.

2 Case Presentations

A 28 years old caucasian male came to Chamran hospital’s orthopedic clinic, Shiraz, Iran with Rt shoulder pain. The patient had pain since 2 years and it became gradually worsen and developed pain on motion and there after limitation of motion. Further, the patient explained night pain about 2 month before surgery.

In physical examination the patient had mild swelling inferior aspect of Rt clavicle at distal 3rd. The range of motion was significantly decreased in all direction accompanied with severe pain. Further, weakness in deltoit muscle was apparent. The neuro vascular examination of Rt shoulder showed upper extremity was intact. The paraclinic lab data which taken recently before the reference showed no abnormality. The Rt shoulder X-Ray showed multiple areas with stippled calcification in and around the shoulder joint which extend to middle 3rd of clavicle and other hand to proximal of humerus (Figure 1). Bone scan suggested mild arthritis in Rt shoulder (FIGURE 1), while MRI screening revealed a significant amount of joint effusion with multiple size hypointense lesion and supraspinatus tendinosis (Figure 2).

Figure 1 X-ray and bone scan of Rt shoulder (increased blood pool and delay uptake) of patient shows synovial chondromatosis.

Figure 2 Axial, coronal and sagittal view of Rt shoulder.
Extensive Synovial Chondromatosis of Shoulder: A Case Study from Shiraz, Iran

Detail diagnose suggested the occurrence of synovial chondromatosis. The patient admitted to Chamran hospital orthopedic center for surgery. Due to extension of osteocartilagenous fragment till middle 3rd of clavicle and till inferior pouch of shoulder joint, we decided to do open arthrotomy of Rt shoulder. The patient underwent operation through deltopectoral approach and evacuation of many fragments and partial synovectomy were done through arthrotomy of shoulder joint (Figure 3).

![Figure 3 Synovial chondromatosis fragments.](image)

After operation rehabilitation started 2 days and patient discharged 4 days after the surgery. The patient followed for 9 month who had full range of motion (Figure 4) without pain with normal imaging.

3 Discussions

Three phases of articular disease have been identified these are

(i) Initial phase which is characterized by the formation of metaplastic cartilaginous nodules in the synovium. This was followed by the.

(ii) Transitional phase characterized by the detachment of these nodules and formation of free intra-articular bodies. The last phase is known as inactive phase in this resolution of synovial proliferation occurred and loose bodies remain in the joint, and may increase in size obtaining nourishment from the joint fluid by diffusion. Similar type of stages was reported by the Miligram (1977).

Chong et al. (2007) suggested that non-steroidal anti-inflammatory drugs can be used along with transcutaneous therapies (eg, ultrasound, thermal therapies) for reduction of inflammation. Patients with primarily mechanical symptoms do not benefit significantly from non operative therapy. Further, Chong et al. (2007) also demonstrated that radiotherapy is a successful modality which can use for synovial chondromatosis of the knee refractory to several previous surgical interventions.

The traditional surgical approach consisted of an open arthrotomy of the joint, with removal of all loose bodies and either a partial or a full synovectomy, but now in these days standard treatment is arthroscopic examination and excision of loose bodies, with limited synovectomy of involved synovium only but local recurrence is not uncommon and occurred only in 3 - 23% cases (Davis et al., 1998b; Murphey, 2007).

Malignant degeneration was also reported in chondrosarcoma but very rarely (Kenan et al., 1993). Additionally, the cellular atypia was also demonstrated as synovial osteochondromatosis and in some instances this may be misinterpreted as chondrosarcoma, and thus a true rate of malignant degeneration is uncertain.

Elmali et al. (2003) studied four patients of synovial chondromatosis, among these two are suffering from shoulder joint synovial chondromatosis. All four patients’ have complaint about the pain in particular organ and restricted joint movement. Two shoulder joint synovial chondromatosis patients, were treated by arthroscopy followed by synovectomy and the loose bodies were removed.

After treatment, all patients became asymptomatic and no evidence of recurrent disease was detected. Result of study suggested that extraction of the loose bodies and arthroplasty are the best treatment for severe osteoarthritis. Similarly, Khorsandi (2007) also studied the two cases of shoulder synovial chondromatosis, both the patients were initially treated with arthroscopic debridement. Later on one patient were went for second arthroscopy and partial synovectomy while the while the second one have treated only by arthroscopy. Result of this study revealed that arthroscopic surgery are better option than the open synovectomy and arthroscopic surgery help the patients to return to normal activity much sooner than a formal open synovectomy. (Khorsandi , 2007). Further, Trajkovski et al. (2011) reported a case of extensive synovial chondromatosis in the right shoulder and surrounding soft tissues with extensive erosion up to the humeral head. These researchers operated combined anterior and posterior surgical excision of the cartilaginous fragments, and described insertion of an osteoarticular allograft to repair the humeral head defect and secondary anterior glenohumeral joint instability and fount better results (Trajkovski, 2011).

A case of synovial chondromatosis which excision of lesion was also reported by (Khandker & Islam, 2010), these researchers also justify the importance of open arthrotomy in the treatment of shoulder joint.

Conclusion

Result of study and available literature suggested that early management of synovial chondromatosis help in the prevention of secondary degeneration of cartilage and help in the enjoy better and pain free life. Occasionally, osteocartilagenous loose bodies are not in higher number but the larger size may cause more severe synovial
chondromatosis. Result of study and literature review suggests open arthroscopy for the treatment of synovial chondromatosis rather than arthroscopic procedure till advanced arthroscopic technique to prevent repeated surgery or recurrence of mechanical symptoms.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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