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Effect of Irrigation on the Content of Cellulose in Proso Millet Stalk (*Panicum miliaceum* L.) in Aydın/Turkey Conditions

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A B S T R A C T

Proso millet stalks are an under-utilized material that remains after the harvest. Given the share of large proso millet stalks in total production in Turkey, more than 8% of stalks could be easily available as a raw material in the fabrication of fuels. Unlike corn, proso millet stalks are not used as green fodder or silage due to the higher cellulose content and smaller green leaves for animal feeding. In this study, the usability of proso millet plant as a biofuel material due to this feature was tested in terms of the chemical parameters such as cellulose, lignin and some other nutritional elements. The impact on the plants grown and harvested materials parameters by applying control (non-irrigated) and four irrigation levels (50%, 75%, 100% and 125%) was investigated. Results of the study show that the highest values were obtained for 125% irrigation level.

1. Introduction

About 20 different millet species have been cultivated worldwide and occupy over 30 million hectares in 30 countries located in Asia, Africa, the Americas and Australia (FAO 2020). Millet is one of the important cereal grains in the world and sustaining more than one-third of the world's population (FAO 2020) and is an important cereal crop for arid and semi-arid climate areas where rainfall is low and erratic (Habiyaremye et al., 2016; Taylor, 2019).

Commonly cultivated millet species (*Panicum miliaceum* L.) are characterized by high tolerance to abiotic stresses such as drought, salinity, high temperatures and nutrient deficiency in the soil (Saleem et al., 2021).

Millet is an annual summer grass and can complete its life cycle within 60–100 days (Habiyaremye et al., 2016). It is a highly nutritious cereal grain used mainly for human consumption (Meena et al., 2021). However, most millet crop in Turkey is used primarily for bird feed and cattle-fattening rations. In 2020, 5.7 million proso millet was produced in Turkey (TUIK, 2021).

Millet grains, as was mentioned before, are used as food or feed for animals, but there are also, after harvesting remained, stalks, which are often considered residue discarded as agricultural waste which can be used for energy purposes.

Using agricultural residue material for the energy holding sector, like biofuels and biogas, has many benefits for farmers and the environment, such as reducing the need for waste disposal, which currently increases farming entry costs and reducing environmental pollution such as fires or pesticides. On the other side, the increasing cost of energy and finite oil and gas reserves has created a need to develop alternative fuels from renewable sources. The need to generate an ample and sustainable supply of biomass to make biofuels generation from lignocellulose profitable will

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require the development of crops grown specifically for bioenergy production as well as the application of agricultural residues (Arevalo-Gallegos et al., 2017; Wzorek et al., 2021).

Lignocellulosic residues (agricultural wastes, forestry residues, grasses and woody materials) are complex carbohydrate polymers built from sugar monomers (xylose and glucose) and lignin – a highly aromatic material. They comprise cellulose, hemicellulose, lignin, extractives and several inorganic materials (Anwar et al., 2014).

It was stated that the composition of lignocelluloses materials/residues varies with species and variety, but also because of the type of soil, weather conditions and the age of the plant (Yadav et al., 2019; Guimarães et al., 2009).

The possibility of using the stalk as a local source of cellulose has not been studied systematically. For example, Packiam et al. (2018) determined that pearl millet corn was rich in cellulose (41.60%), hemicellulose (23.32%), and lignin (21.81%). In contrast, pear millet stalks contain 52.49% cellulose, 25.42% hemicellulose and a relatively low level of lignin, 10.54% (Yadav et al., 2019). Therefore, it looks like millet stalks are a promising feedstock for the energy sector.

While the producing reason of bioenergy feedstock is to produce renewable fuel, one of the critical components in their production will be water. According to one of the new types of irrigation strategies, deficit irrigation scheduling is a valuable and sustainable production strategy which can be applied to dry regions (Yilmaz et al., 2021).

Specially millet varieties need relatively less water than the other crops because they have short growing seasons and show exceptional tolerance to various abiotic and biotic stresses (Taylor, 2019).

Some studies explained this impact of water stress; for example, Seghatoleslami et al. (2008) observed that deficit irrigation for three species of millet (*Panicum miliaceum*, *Setaria italica* and *Pennisetum americanum*) declined yield by reduction of seed number per panicle and panicle number per plant and caused reduction in the number of tiller, as well as peduncle and panicle length and plant height. Yadav et al. (1999) demonstrated that water stress after pollination in pearl millet reduces seed yield by reducing the number of tillers per m^2, number of seeds per panicle and seed weight. Bartwal et al. (2016) obtained a 200% increase in ascorbate content, limiting ROS accumulation (Reactive Oxygen Species) for finger millet. Golombek & Al-Ramamneh (2002) indicated that drought reduces CO_2 assimilation area by reducing leaf number and area.

The water-use efficiency is critical as one of the factors for plant production where rainfalls and water reserves are limited.

There is little data on how irrigation affects stalk lignocellulose concentrations. Hence, the main idea of this study is to analyze the effects of irrigation on stalk lignocellulose concentrations in proso millet stalks. Also, this study can provide valuable information for proso millet stalk potential as a biofuel feedstock.

2. Materials and Methods

In this study, a local variety of proso millet (*Panicum miliaceum* L.) was used as plant material.

Local and climatic descriptions of the experimental area

The experiment was conducted between the end of May and the beginning of September 2020 at the Department of Biosystems Engineering research center and application on the field of Faculty of Agriculture Farm, which is located on the south campus of Aydın Adnan Menderes University (N:37º 45’ 39”; E:27º 45’ 37”), in town Koçarlı of city Aydın in Turkey (Figure 1.)

Table 1 presents the long-term averages of the selected meteorological data study area.

Figure 1. Location of Biosystems Engineering research area.

Table 1. shows that in 2020 during sowing time (May), the temperature of the air was 1.1 ºC higher than in the period of 1970-2019, the humidity was 2% less, as well as rainfall, but the level of evaporation was higher compared to

previous years. All parameters were higher than normal except for the temperature ratio in June and July. In August, temperature and evaporation ratios were higher than normal, and rainfall and humidity were less than normal. Generally, it can be stated that the climatic conditions were changing, which is not suitable for plant growth.

Table 1. Long-term averages selected meteorological data comparing to the year of study.

1970-2019				
Month	**Temperature (ºC)**	**Humidity (%)**	**Rainfall (mm)**	**Evaporation (mm)**
May	21	56.9	35.6	161.3
June	26	49.2	16.6	222.1
July	28.6	48.6	7.5	257.5
August	27.6	52.9	5.3	231.6
September	23.3	55.9	15.1	161.9
2020				
Month	**Temperature (ºC)**	**Humidity (%)**	**Rainfall (mm)**	**Evaporation (mm)**
May	22.1	54.9	33.3	175.2
June	25.2	54.4	20.3	200.2
July	29.9	47.8	0	272.6
August	29.2	46.9	0	247.1
September	26.9	54.7	0	182.8

Soil Description

Soil profiles of the study area are presented in Table 2. Classification of soils of the research area is sandy-loam type in effective root zone sandy-loam. Moreover, the volume weight of the soil profile by irrigation norms is in sequence 1.35, 1.45, and 1.52 g cm^{-3}. The available water capacity of soil by irrigation norms was changing by deep of soil in 300 mm in sequence 52.7, 58.7, 50.6 mm.

Table 2. Soil profiles of the study area

Soil Profile deep (cm)	Structure of soil (%)			Classification	Volume weight (g cm^{-3})	* Field capacity		* wilting point		Available water capacity	
	Sand	Clay	Loam			(%)	(mm)	(%)	(mm	(%)	(mm)
0-30	58.4	13.6	28.0	Sandy loam	1.35	23.1	93.6	10.1	40.9	13.0	52.7
30-60	56.4	13.6	30.0	Sandy loam	1.45	22.9	99.6	9.4	40.9	13.5	58.7
60-90	68.2	13.6	19.2	Sandy loam	1.52	18.4	83.9	7.3	33.3	11.1	50.6

Methods

Experimental layout, design and treatments

This study was carried out in three replications in a randomized block design with 5 different doses of water (50%, 75%, 100% and 125% and control (non-treatment)). The soils of the trial area were plowed with a plow after the first spring rains, and the formed clods were fragmented with a discarrow, and the seedbed was prepared by passing a rake and a slide. Afterwards, the seeds were sown with a pneumatic seeder to a depth of 5 cm, with 4 rows which contains as distance 70 cm from each other and at 10 cm above the row. The plot lengths were marked as 5 m after vegetative growth started and while plants were 15-20 cm high the proso millet plants were ejected with a discarrow between the blocks as 3 m and between plots 2.1 m spaces.

Irrigation water

Irrigation water doses were calculated using Cropwatt version 8.0 software by 7 days intervals.

Irrigation topics were designed for deficit irrigation conditions, which topics were informed before, and the pipelines were designed as main lines with 75 mm diameter surface (PVC), which transport irrigation water from the control unit to 3 manifolds which are controlled by valves as 63 mm diameter (PVC) and from manifolds for control the flow by manual PVC valves to the 16 mm diameter drip irrigation lateral lines (PE), which contains inline drippers with included pressure controller membrane and 2 l/h flow and above the row 20 cm. Evapotranspiration ET0 ratios were calculated by equation 1 (Walker and Skogerboe 1987).

$$ET = I + P + Cp - Dp \mp Rf \mp \Delta S \quad (1)$$

where: ET - plant water consumption (mm), I - over the period amount of irrigation water applied (mm), P - rainfall over the period precipitation (mm), Cp - the amount of water entering the root zone by the capillary rise (mm), Dp - penetration losses (mm), Rf - the trial amount of runoff (mm) entering and leaving plots of land, DS - root changes in soil moisture in the region (mm), values shows. Since there is no groundwater problem in the study area, there is no capillary water entry into the plant's root zone. Assuming the Cp value is not taken into account. Also, the surface flow amounts were not included in the accounts because the pressurized irrigation system was used (Kanber, 1997). Penetration losses of a substrate were observed for defemination of the level of penetrating deep.

Harvesting procedure and data collection

The harvesting of samples of plants was collected at the end of August and the beginning of September. The plant rows located on the border of parcels were separated and samples were measured and taken randomly into two rows which were in the middle of parcels and biomasses were calculated by using parcels size for per decare (1.40 m x 4.80m = 6.72 m^2).

Sample preparation for chemical analysis

Raw materials were cut into 3–5 cm segments and prepared to be dried. The moisture content was determined according to ASTM D 2216. The percentage difference between the initial weight (1 g) and the final weight of the sample was calculated after three days drying them at 60 °C to a constant weight. The sample was allowed to cool in a desiccator and reweighed to record the final weight. Samples were tested in triplicate, and the average of three values was recorded. The moisture content of the material was calculated using the following equation 2.

$$\text{Moisture content} = W1 - W2/W1*100 \qquad (2)$$

where: W1 is the weight of the moist sample, and W2 is the weight of the dry sample.

After drying, samples were milled and sieved to obtain 1 mm particles.

NIR spectroscopy

After completion of the treatments, all samples were analyzed using near-infrared spectroscopy (NIRS) which is a rapid analysis method that is expanding the application field. The applications of NIRS in forage have long been investigated by, i.e. Ono et al. (2003) Chen et al. (2006), Huang et al. (2010) and Guo et al. (2021) and shows successful results for the analysis of the composition of plant materials.

Absorbance measurements were made using a near-infrared reflectance spectrophotometer (Bruker MPA analysis) which was connected to a personal computer. The computer was equipped with software (OPUS Lab). This device covered a spectral range of 400–2500nm in 2nm intervals.

All samples were analyzed to determine chemical composition. Parameters such as NDF (neutral detergent fiber), ADF (acid detergent fiber), and ADL (acid detergent lignin).

Each sample was analyzed in triplicate, and the results were expressed as weight percent on a dry basis. The cellulose, hemicellulose, and lignin of each sample are related to NDF, ADF, and ADL by the relation (Van Soest and Robertson, 1985):

ADL = lignin and cutin
ADF = lignin and cutin + cellulose
NDF = lignin and cutin + cellulose + hemicellulose

Hemicellulose was calculated as NDF -ADF.

Statistical analyses

The descriptive statistics, including the minimum, maximum, mean, SD, ANOVA and LSD tests, were calculated by SPSS and TARİST software.

3. Results and Discussion

Water Use Efficiency (WUE)

Ullah et al. (2017) argue that the optimal water requirements for pearl millet precipitation range from 300 to 350 mm. His experiments with pearl millet showed that growth and development respond to good soil moisture conditions (irrigation) and even function reasonably under unpredictable weather conditions due to deep and rapid root penetration. Pearl millet requires less water than other crops; for example, sorghum, wheat, corn, cotton, rice and sugarcane require 14, 28, 42, 71, 57 and 500% more water than pearl millet, respectively.

Some field studies compare water stress for millet and other crops to determine the water use efficiency (WUE) and yield response (Hulse et al., (1980); Maman et al., (2003); Murthy (2007). For example, Maman et al. (2003) compared pearl millet and soybean in the western Nebraska region in two seasons, 2000 and 2001 and for both crops were used a similar amount of water (was 336 and 330 mm in 2000 and 370 and 374 mm in 2001 for pearl millet and grain sorghum, respectively). Both crops responded to irrigation with a linear increase in grain yield as water use increased. Pearl millet grain yields were 60 to 80% that of grain sorghum.

Singh and Singh (1995) have reported that the plot level and water use efficiency (WUE) values of 300–400 kg biomass ha^{-1} cm^{-1}, assuming a full ground cover (LAI > 3–4). However, under low planting density, the WUE usually drops to the range 50–150 kg ha^{-1} cm^{-1}, primarily because of an increased evaporation component (Payne, 1990), itself high because of the fertility-related low sowing density. Therefore, it seems that fertility may be the number one factor to improve the WUE at the plot level.

In most of the cases, high yield and WUE were noted under full irrigation, indicating the high potential of pearl millet crop (Nagaz et al. 2009)

The impact of water on the transpiration rate is also observed (Tr). Vadez et al. (2012) observed that it sets the maximum amount of carbon dioxide with water availability. Furthermore, the very availability of water forces the stomata in such a way that the transpiration efficiency (TE) can be kept at the highest possible level. Physiologically, plants show low photosynthetic and stomatal conductance rates and root respiration which help them to escape from water stress (Taylor 2019).

Table 3 presents parameters such as biomass, irrigation, seasonal evapotranspiration, irrigation water use (IWEU) and water use efficiency (WUE).

Irrigation water ratios were between 235 mm to 587.5 mm, and seasonal evapotranspiration levels were changed between 215.6 mm and 641.1 mm. The ratios of IWUE were calculated as 2.24 kg m^{-3} to 6.14 kg m^{-3}, and WUE was calculated in sequence between 2.05 kg m^{-3} and 5.7 kg m^{-3}.

Calculated data shows how much mm water will applicate by drip irrigation for full irrigation topics; the other research topics were used as the ratio in raw 1.25, 1.0, 0.75, 0.50 and 0 (control). The yield ratio of proso millet stalks was changed between 1233.33 and 1853. 33 kg da^{-1}.

As shown in Table 3, the biomass ratios were changed parallel by the level of irrigation water. Moreover, the biomass ratio was less on level 1.25 irrigation coefficient when compared with a parallel decrease of irrigation levels. It can be stated that the cellulose content of stalks was negatively affected by higher irrigation levels of water. Fibrous construction, which was determined in proso millet stalks, can influence on less irrigation levels more than the full one. It also explains that biomass on each tested irrigation level was less obtained than the full irrigated level.

Chemical components

From the point of view of energy use of biomass, three substances are the carrier of most of the energy accumulated in biomass: cellulose, hemicellulose and lignin. Some research also indicated a high correlation between calorific value and the content of cellulose, hemicellulose, and lignin (Demirbaş, 2005). Ultimately, the heat of combustion of biomass depends mainly on the percentage share of cellulose, hemicellulose and lignin in its structure, but the decisive factor for the high value of the heat of combustion is, therefore, the share of lignin in relation to holocellulose.

Table 3. Biomass, irrigation, seasonal evapotranspiration, irrigation water use and water use efficiency

Treatments	Biomass (kg da^{-1})	Irrigation (mm)	SET Seasonal ET_o (mm)	IWUE (kg m^{-3})	WUE (kg m^{-3})
125%	1316.67	587.5	641.1	2.24	2.054
100%	1853.33	470.0	523.6	3.94	3.540
75%	1730.00	352.5	406.1	4.91	4.260
50%	1443.33	235.0	288.6	6.14	5.000
Control (non-treatment)	1233.33	0	215.6	0	5.700

In Table 4 are presented the average values of cellulosic components as cellulose, hemicellulose, and lignin and also NDF, ADF, and ADL after subjecting to different water stress.

Table 4. Chemical characteristics of proso millet stalk (in dry base)

Average %					
Level of irrigation	**125%**	**100%**	**75%**	**50%**	**0**
Nb of samples	**1**	**2**	**3**	**4**	**5**
Cellulose, %	37,40	37,35	36,64	35,97	34,20
Hemicellulose, %	30,28	25,90	19,72	19,23	17,85
Lignin, %	12,24	11,68	11,56	10,34	8,90
NDF %	67,30	62,18	58,87	58,22	55,09
ADF, %	37,02	36,28	39,15	38,99	37,24
Dry mass, %	94,24	92,90	94,73	93,86	95,09
Moisture, %	5,76	7,10	5,27	6,14	4,91

The cellulose content in millet stalks raged from 34% to above 37% and was similar content as was observed by Harinarayana et al. (2005) - 39.4%. Regarding the level of irrigation, it can be seen that the cellulose content decreases as the amount of irrigation water fed decreases as the plant grows.

Compared to other non-woods, millet has similar cellulose content of corn stalks 32.4% (Siriwattana, 2002), tobacco stalks (Burley) 34 % (Kulic and Radojc, 2011), rice husks 36% and cotton stalks 38% (Singh and Chouhan, 2014).

Hemicellulose content in millet stalks raged from 17% to 30%, whereas lignin content is in the range 8-12%. Huang et al. (2010) and Wzorek and Troniewski (2007) clam the average content of lignin in stalk-type biomass is 15-16 wt%.

Lignin content in grasses depends mainly on the degree of lignification, which is related to the maturity of the shoots. Growing shoots contain less lignin, while mature, dying shoots contain more of it, because the lignification process reaches its maximum just before cell death.

Yadav et al. (2019) claim that the lignin component (acid soluble and insoluble) is maximum (29%) in the sheath and lower (9–10%) in the core and outer leaves. It is because the lignin present in the cellular wall (sheath) provides structural support, impermeability and resistance against microbial attack and oxidative stress.

Also, there were no statistically significant relations on the Anova test between water level and cellulose ratios as linear (Table 5). However, results showed that there are exponential relations between the cellulose content of proso millet stalks and irrigation levels.

Figure 2 are presented samples obtained from different irrigation levels.

Proso millet is one of the low-water consumption crops. Research has shown that prolonged periods of warm and dry weather can be detrimental to it and can reduce yields.

Figure 2. Full irrigated and no-irrigated stalk of proso millet

Table 5. Results of the content of chemical and Anova LSD analysis

	Source of Variation	Degrees of freedom	Sum of squares	Mean Sum square	Calculated F	Sequence LSD results
PROTEIN	Repetition	2	0.348	0.174	0.932ns	4,5,1,3,2
	Factor A	4	0.623	0.104	0.556ns	10.237-10.233 10.070-9.917 9.847
	Error	8	0.241	0.187		
	General	14	3.212	0.161		
FAT	Repetition	2	0.804	402.175	1.018	1,3,3,2,4,5
	Factor A	4	2202.8	367.133	0.930ns	32.817-7.350 6.560-3.667 2.793
	Error	8	4738.628	394.886		
	General	14	7745.777	387.289		
Starch	Repetition	2	0.186	0.093	0.105ns	3,2,5,1,4
	Factor A	4	2.817	0.469	0.530ns	10.37-9,860 9.633-9.510 8.993
	Error	8	10.629	0.0886		
	General	14	13.631	0.682		
Ca	Repetition	2	0.330	0.165	0.235	5,4,3,1,2
	Factor A	4	2.046	0.341	0.485	1.630-1.080 0.927-0.897 0.767
	Error	8	8.441	0.703		
	General	14	10.818	0.541		
Mg	Repetition	2	0.001	0.001	0.143ns	2,3,1,4,5
	Factor A	4	0.010	0.002	0.411ns	0.240-0.237 0.207-0.197 0.197
	Error	8	0.048	0.004		
	General	14	0.059	0.003		
Biomass	Repetition	2	21.532	10.766	1.692ns	1,4,2,5,3
	Factor A	4	29.699	4.950	0.778ns	94.920-94.733 94.240-93.857 92.897
	Error	8	76.379	6.364		
	General	14	127.600	6.380		
Cellulose	Repetition	2	1.688	0.844	0.670ns	1,2,4,5,3
	Factor A	4	4.446	0.741	0.589ns	37.867-37.293 36.687-36.653 36.587
	Error	8	15.106	1.259		
	General	14	21.239	1.062		
ADF	Repetition	2	66.285	33.143	0.462ns	5,3,4,1,2
	Factor A	4	900.237	150.040	2.090ns	45.160-39.573 35.100-29.383 24.093
	Error	8	861.459	71.788		
	General	14	1827.982	91.399		
ADL	Repetition	2	3.177	1.589	0.368ns	5,2,1,3,4
	Factor A	4	8.563	1.427	0.330ns	12.400-10.953 10.740-10.730 10.623
	Error	8	51.858	4.321		
	General	14	63.598	3.180		
NDF	Repetition	2	75.807	0.535ns		5,3,4,1,2
	Factor A	4	1023.351	2.409ns		57.607-50.350 45.770-40.153 35.093
	Error	8	849.581			
	General	14	1948.740			

ns: non-significant

4. Conclusions

Proso millet stalks can be an attractive biomass source for energy applications. However, due to problems with water resources, the amount of water introduced into the soil during its growth phase should be optimized.

Tests show that the influence of irrigation on the content of cellulose, hemicellulose and lignin in the samples of proso millets stalks increased at different water content; it can be concluded that there was a tendency of the increase of these parameters along with the amount of water administered during their growth. The highest values were obtained for the 125% irrigation level. In this study, irrigation water was changed between 235 – 587.5 mm in Aydin condition, and seasonal evapotranspiration was determined to the topics between 215.6 mm and 641.1 mm.

IWUE were determined in these conditions from 2.24 V to 6.14 kg m^{-3} and WUE were determined as between 2.05 kg m^{-3} and 5.7 kg m^{-3}.

In this study, there were no statistically significant relations on the ANOVA test between water level and cellulose ratios as linear; however, results show that there are exponential relations between cellulose content of proso millet stalks and irrigation levels.

It can be stated that the cellulose content of stalks was negatively affected by higher irrigation levels of water.

Fibrous construction determined in proso millet stalks was more influenced by less irrigation levels compared to higher irrigation levels. Results also explain that biomass on each tested irrigation level was less obtained than full irrigated.

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