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Research Article

Carbon Sequestration and Biomass Assessment of Mott Grass (*Pennisetum purpureum*), in three Growth Stages in Barani Areas of Pothwar, Pakistan

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Abstract | Alternative renewable energy is getting progress in current era keeping in view of energy insecurity in the coming years and the ever increasing threat of environmental and social health due to the use of fossil fuel. Lingo-cellulosic biomass has seen getting fame as bioenergy crops in maintaining food security alongside fulfilling energy demands. Napier grass (*Pennisetum purpureum*) has turn out the promising nutritious forage to the livestock on the sustainable basis, feedstock for biofuel and also has proven to be environmental friendly. The purpose of this study is to assess the biomass production of mott grass and its capability to sequester Carbon from the environment in the barani areas of Pothwar region in Pakistan. The research study was conducted under rained condition in the field area of Rangeland Research Institute National Agricultural Research Centre Islamabad. In this regard an experimental plot of one acre area was established in the field area of RRI, NARC, Islamabad. Grass tuft of sized 15 cm was collected from the grass nursery of range land Research Institute (RRI) and plantation was carried out in one acre in barani condition. The area was laid down and plant to plant distance was 60 cm and row to row distance 90 cm kept in the research study area in RRI, NARC. The parameters of this experiment were included biomass production (BP) kg/ha, carrying capacity (CC) kg/ha and carbon stock (CS) kg C /ha after interval of 21, 45 and 60 days along with the proximate analysis including protein content, crude fiber, ether extract, and total digestible nutrients. Significant results were obtained by applying ANOVA test on the data retrieved. All three factors (BP, CC and CS) showed maximum results after 60 days of growth. The result indicated that after the 60 days interval the animal unit per month gave the maximum fodder (7.0667 AU/M/Ha) that proposed to be grazed for large ruminants and then 21 small ruminants on the same biomass and also maximum forage production recorded 3882 kg/ha and it sequester carbon to up to 3560 kg C/ha in the given interval. Increase in fiber and ash content also justify the increase in biomass yield of the plant after interval of 60 days. The study indicates that Mott grass or Napier grass based on its high biomass yield is an effective approach in maintaining the fodder quality for animals on the sustainable basis and being bioenergy crop could make it a viable asset in declining the carbon burden off from environment and fulfilling the upcoming energy demands.

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Introduction

Grass plants create most extensive part of range vegetation. Usually grasses are more easily manageable; better in taste and quicker in digestion than shrubs and trees for grazing and browsing animals (Sarwar *et al*., 2005). As compared to any other cultivated plant the grasses have highest potential of starch and protein. In the 60 years of livestock development in Pakistan, it experience the most critical situation today. Energy issues of the country and price hike have consequently increased the price rate of animal food. This has aggravated the societal and environmental problems of food production. Owing to all these scenarios livestock food production is among the highlighted research and development area of the country. As the production potential of the rangeland have been reduced to minimum in few years, grasslands are required to be replenished in order to enhance the standard of grazing (Afzal *et al*., 2007). Presently, Pakistan is declining in livestock food deficiency by more than 21 percent (Qamar *et al*., 1999). It is the time now to enhance the yield of forage per hectare and meet the need of animals throughout year on sustainable basis by providing good quality nutritional food through grasses. Rangelands are the major source of forage for livestock, are declining by facing adverse weather condition and over grazing particularly in Pakistan. Increase in the human population there is also a rise in protein demand from animals hence adding more stress on the rangelands in the country. Rangelands to be managed and treated properly requires the extensive knowledge of forage calendar, their nutritional potential, forage delectableness and preference of the livestock in a certain region. According to an estimated research, total digestible nutrients and crude proteins are declining by 29% and 33% respectively in already available feed sources. Whereas, total dietary intake is linked to the animal forage digestibility, nitrogen amount and fiber. The protein content 6-8% are in the forages makes it digestible for ruminant microbes (Styler *et al*., 1979).

Pennisetum purpureum is a perennial grass species located in the tropical and subtropical areas, Mott, Napier, Elephant and Uganda grass are all common names of this same species. It is native to Tanzania. It has tall, erect and thick stems of 4.5 m height. This grass is known to high biomass production with excellent growing ratio (Wang *et al*., 2002). This grass was first introduced in the North America in 1913 as a fodder (Thompson, 1999). Originally available in Brazil is a source of fiber and have the capability to grow in any soil type where fiber content can reach up to 40% in quantity (Ade-Araujo *et al*., 2013). Napier grass is good in producing high value yield and increased growth rate while having ability to withstand negative environmental conditions. Good agronomic feature and effective dietary food makes it a useful nutritional resource. Whereas it is used less as food source due to limited research about its nutritional benefits (Chang and Muzi, 2009). In livestock farming Mott grass is responsible for the production of good quality forage and also recommended for silage. According to research carried out in 2018, Brazil only generated 30–40 metric tons of its biomass per hectare under home condition while other areas produced 50-60 MT in five months using rectilinear growth rate (Danquah *et al*., 2018).

Pakistan also is in queue in yielding high quality fodder varieties that continues to maintain its worth during regrowth periods. This fodder has an advantage of being available in slump period (May-July) when other fodders are limited. The availability of Mott grass could enhance the quality of fodder supply on sustainable basis to farmers during the lean period, and could be used in second decline duration January to March, depending upon the eco types in the country when again fodder quantity is reduced to minimum (Bilal, 2001). Reduced quantity of fodder and ultimately low quality effects animal production in developing countries like Pakistan. It is of the view that in near future, due to exponential increase in the population humans will be in direct competition with animals for edible purpose and hence there will be more dependency on forage which will ultimately put stress on the fodder accessibility. According to research only 3.35 million hectare of land is devised for fodder crops out of 22.96 million hectare while remaining is agriculture for other crops. This area of 3.35 million hectare is producing 58 million tons of fodder with the whole area declining at the rate of 2% every decade (Gill, 1998). Having Mott grass in the country add another benefit of using this biomass for energy production. Grasses and wood have unique cellulosic property that can generate heat and electric power by direct combustion in the environment or it can either be used as liquid transportation Moreover *Pennisetum purpureum*, being short rotation crop can sequester carbon from the environment and help ecosystem to replenish its quality (Moore *et al*., 2008).

The purpose of this study is to determine the above ground biomass content of the *Pennisetum purpureum* grass species by comparing the biomass yield in its three growth stages with their carbon sequestration in three growth stages under rain fed condition in the Pothwar region of Pakistan.

Materials and Methods

Study area

The study was conducted in the field area of Rangeland Research Institute (RRI), National Agriculture Research Center (NARC) Islamabad. Islamabad is situated at 33.43 N and 73.04 E on the edge of Pothwar plateau at the end of Margalla Hills in Islamabad at about 507 meters altitude. It lies in the moist, hot subtropical climate and humid summers, monsoon season followed by cool winter. The soil is locally outwash/loessic, alluvial in the source. It is mildly calcareous and content of lime is equally dispersed in complete contour of soil. Soil in this region are not sodic or saline which has little basic pH with minor amount of inorganic substances. It contains less organic material that ensure 7.5-8.5 pH (Ahmad *et al*., 2001). Rain fall is quite irregular which differs significantly from 250 mm in south-west to 1000 mm in the north-east areas. During the summer months more than 70% of annual rain falls occur.

Study design

An area of one acre was selected, ploughed, cleared form every kind of debris. Then the area was laid out. Planting was carried out with the stumps of Mott grass in the field area of RRI, NARC, Islamabad in Pothwar. Row to row distance was kept at 90 cm and plant to plant distance was retained at 60 cm. It was plantation of experiment was grown with the stumps. The length of the stumps was cut down to 15 cm maximum having more than two buds. The line with help of drill was manually dug out up to 8-10 cm in depth (Figure 1).

This one year cutting of Mott grass was buried horizontally into soil, when soil was holding sufficient moisture in beneath. After sprouting, the Mott grass was irrigated initially to planted stumps then the sample was irrigated as and when required. For the production potential and carbon sequestration analysis, the first cutting of Mott grass was obtained at 30-45cm height on 20-25th day. The one acre area was divided into three compartments. Hence, the total quadrates were nine in one acre area. In each compartment there were three quadrates were taken randomly. One meter square implement (quadrate) was used for biomass production for kg/ha. Inside the quadrate all the grass was cut down and put into bag and marked. Then took their fresh weight was measured at the spot for fresh production for kg/ha. Then brought into laboratory and dried at oven up to 60-70 degree centigrade for three days until constant weight. The dry weight was taken after the grass was fully dried for kg/ha production. The total biomass production was then multiplied with a universal coefficient factor 0.50 for carbon sequestration. The same procedure was adopted for second and third cutting done in next 40-45 days and 60-65 days respectively, for biomass production and carbon pool. Similarly, same methodologies was repeated for the second and third data for forage production potential in kg/ha and also for carbon stock in lab of NARC.

Chemical analysis

The proximate analysis (protein, ash, ether, and fiber contents) of Mott grass samples at three stages was conducted in Animal Nutrition Lab, NARC, and Islamabad using AOAC, 2000 standard method.

Dry matter

5 mg of sample was collected in already weighed crucible and was weighed again to calculate dry matter. The sample is placed overnight at 100 degree Celsius. After this sample containing crucibles are kept is desiccator for 20 to 30 minutes and weight again which is the overall amount of dry matter obtained.

$$DM\% = \frac{Weight\ of\ dried\ sample}{Weight\ of\ fresh\ sample} \times 100$$

Estimation of crude protein

Kjeldhals technique is used to analyze the crude protein content in the sample. 5 mg sample was taken with 3 mg of potassium sulphate (93%), copper sulphate (7 %) and 5 ml of concentrated sulphuric acid (75%) and digested. In digestion tube 5 ml of diluted sample along with 7ml of Na OH is taken and distillation of sample is done for 7 minutes. The ammonia gas produced as a result of this reaction is mixed with in 2 percent of H3BO3 solution in a flask. Methyl red were used as an indicator. Ammonia is titrated against sulphuric acid using titration method and blank samples were repeated for whole experiment.

Calculation of % nitrogen is done using:

$$N\% = \frac{((V1 - V2) \times 14.01 \times 0.02 \times D)}{Weight\ of\ sample} \times 100$$

Where,
V1= Sample titration; V2= Blank titration; 14.01= Atomic weight of N; D= Dilution factor

For mott grass additional neutral detergent fiber and acid detergent fibers were calculated. For this 2g sample is taken and refluxed with NDF and DDF solution Van-Soest *et al* (1991). NDF analysis is done without using sodium sulfite and α–amylase as the mott grass is mature which contain very little NDF and starch in this stage could interfere with fiber content. NDF and ADF were presented exclusively.

Ash estimate

Properly dried sample were burnt in muffle furnace at 550 °C temperature for the duration of hours. Sample were placed in desiccator for 20 to 30 minutes after burning. The result obtained gives the actual amount of ash in sample.

Ether extraction estimate

5mg of 1mm particle size of grass sample is taken on man, s filter paper for the estimation of the ether in Mott grass. Filter paper was wrapped around the sample and is shifted to thimble in extractor. Extraction assemble is made attached with dried ether flask filled with petroleum ether (1/3). On the disappearance of petroleum ether from carafe, extraction assembly (condenser and heater) were changed showing dense state and was then dropped in thimble. Resulting products were again tapped in the receiving flask. After completion flask were taken to water bath (100°C) and oven dried at 100 °C till ether is removed successfully.

$$Ether\ Extract\% = \frac{weight\ of\ ether\ extract}{Weight\ of\ sample\ (g)} \times 100$$

Kjeldhal technique from AOAC (1999), (method no. 976.06) is used to determine the amount of nitrogen in mott grass. This obtained nitrogen is converted to crude protein by multiplying % N with 6.25. UV spectrophotometry is used to determine phosphorous concentration in the sample. Potassium and calcium were determine using flame photometry method.

Crude fiber

1 g sample is taken in a clean beaker diluted with 200ml of H_2SO_4 (approx. 1.25%) to determine the total amount of fiber in the Mott grass. Sample are taken into weighed porous crucibles digested in crude fiber extraction instrument. After digestion samples are filtered with suction pump and washed with hot water at 100°C. The acids were removed and again diluted with boiling 200ml of NaOH followed by digestion for again 30 minutes. Sample were filtered again after cooling. Further on the sample are washed with diluted acid of 10ml as well as with water at 100°C. After this the dried sample at 100°C in oven are placed in oven for 30 minutes. The desiccated sample is shifted to muffle furnace for ignition. Sample are cooled and weighed (W_2) again after been in desiccator for 30 minutes. The percentage crude fiber is measured as:

$$CF\% = \frac{W2\ - W3}{W1 + (Weight\ of\ sample\ )} \times 100$$

$$FF\%\ (DM) = CF\%\ in\ sample \times 100$$

Where,
DM% in sample: W_1= Empty crucible weight; W_2= Crucible weight + Dried residue p; W_3= Crucible weight + ash residue.

Statistical analysis

Total analysis was conducted at Lab of RRI, NARC. Coefficient of 0.50 was used for the conversion for above carbon (Farooq *et al.*, 2017). The collected data was set and analyzed under randomized complete block design.

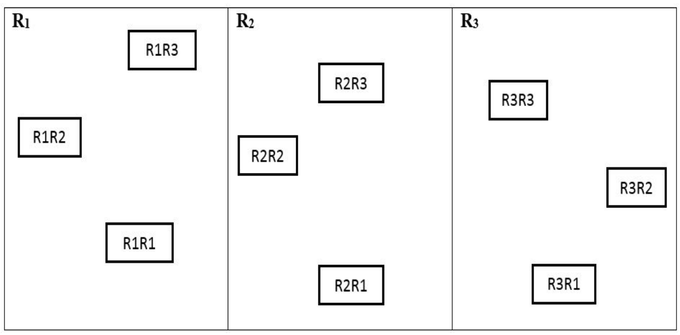

Figure 1: *Figurative explanation of experimental plot.*

Results and Discussion

The study of carbon sequestration and biomass assessment of Mott grass in three growth stages in barani areas of Pothwar, Pakistan was conducted in the field area of Rangeland Research Institute (RRI), National Agriculture Research Center (NARC) Islamabad. Grasses were grown in nine quadrates for the extensive period of one year to ensure its better growth and proper analysis of carbon stock and biomass generated. The analysis of carbon stock (CS), carrying capacity (CC) and dry weight (DW) was done using following formulas:

$$\text{Carbon stock} = biomass \times 0.5$$

$$\text{Carrying capacity} = \frac{allowable\ food}{9.1 \times 30}$$

$$\text{Dry weight} = \frac{total\ dry\ weight\ gm/m2}{total\ no.\ of\ quadrants}$$

Table 1 represents the data collected from experimentation unit to measure dry weight, carrying capacity and carbon stock content of Mott grass for the duration of 21, 45 and 60 days. The average LSD limit was calculated against factor $P< 0.05$, and with average values of 304.53, 0.4502, and 0.1466 for dry weight, carrying capacity and carbon stock, respectively.

Table 1: *Values obtained for dry weight, carrying capacity and carbon stock estimation.*

Time (days)	Dry weight kg/ha)	Carrying capacity (AU/ha)	Carbon stock (Mg C/ha)
21	3388.9b	6.1667b	3.0833b
45	2836.7c	5.2000c	2.6467c
60	3882.0a	7.0667a	3.5600a
LSD 0.05	304.53	0.4502	0.1466

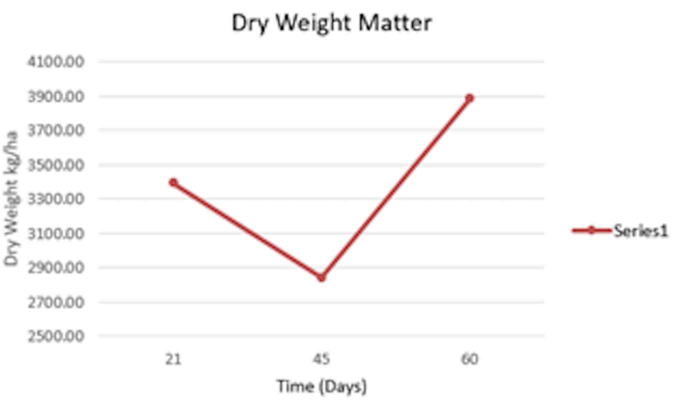

Figure 2: *Dry weight content of mott grass*

Figure 2 shows the values obtained for dry weight. According to the data obtained the dry weight matter of the moot grass was increased with the increase in the interval of sowing and time limit of the grass into the soil. Date shows the higher DW limit at 60 days interval as compared to other days. Similarly, the same trends were shown for Carrying capacity and carbon stock of the Mott grass in the experimental area Figures 3 and 4, respectively.

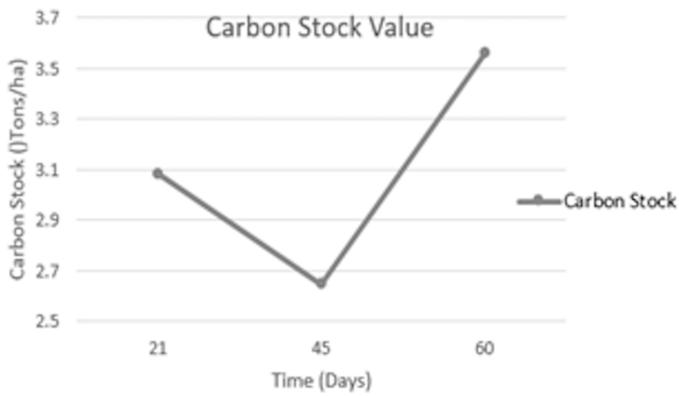

Figure 3: *Carbon stock of Mott Grass.png*

Figure 4: *Carbon stock of Mott Grass*

Statistical data results

Randomized complete block design for dry weight is given in Table 2. Grand mean for all the values taken is 3369.2 for every rep and time and critical value come out to be 3.99 for dry weight.

Similarly, the Table 3 for carrying capacity of mott grass highlights the grand mean to be around 6.1444 whereas the critical value is 3.23.

ANOVA test applied for carbon stock of the Mott grass depicts the grand mean of 3.0967 against 2 repetitions. The critical value given in Table 4 is 2.09 for total carbon content. The P-Value for all the results carried out through ANOVA ($P< 0.05$). The result indicated that in Table 5 there were significantly difference in all means from one another during the three intervals of dry weight. In Table 6 all the means were significantly different from one another in all three stages biomass carrying capacity. The result indicated in Table 7 that all the means of carbon stock in all three stages were significantly different from one another.

Table 2: *Randomized complete block AOV table for DW.*

Source	DF	SS	MS	F	P
Rep	2	2357228	1178614		
Time	2	1640831	820416	45.46	0.0018
Error	4	72182	18045		
Total	8	4070241			
Grand mean: 3369.2		CV: 3.99			

Table 3: *Randomized complete block AOV table for CC.*

Source	DF	SS	MS	F	P
Rep	2	8.6956	4.34778		
Time	2	5.2289	2.61444	66.28	0.0009
Error	4	0.1578	0.03944		
Total	8	14.0822			
Grand mean: 6.1444		CV: 3.23			

Table 4: *Randomized complete block AOV table for CS.*

Source	DF	SS	MS	F	P
Rep	2	2.18540	1.09270		
Time	2	1.25207	0.62603	149.65	0.0002
Error	4	0.01673	0.00418		
Total	8	3.45420			
Grand mean: 3.0967		CV: 2.09			

Table 5: *LSD all pairwise comparison test of DW for time.*

Time	Mean	Homogenous group
3	3882.0	A
1	3388.9	B
2	2836.7	C
Alpha 0.05	Standard error for comparison 109.68	
Critical T value	Critical value for comparison	
Error term used: rep*time, 4 DF		
All 3 means are significantly different from one another		

Chemical analysis

Proximate analysis composition based on the dry matter of Mott grass under the study is carried out in RRI, NARC, Islamabad. All the results are presented in Table 8. According to the data retrieved a gradual drop can be seen in protein content, ether extract, Nitrogen free extract and total digestible nutrients after 60 days. Whereas crude fiber content of the Mott grass was increased from 31.41 to 33.40% by DW in the timeline of 21 to 60 days.

Table 6: *LSD all pairwise comparison test of CC for time.*

Time	Mean	Homogenous group
3	7.0667	A
1	6.1667	B
2	5.2000	C
Alpha 0.05	Standard error for comparison 0.1622	
Critical T value	Critical value for comparison 0.4502	
Error term used: rep*time, 4 DF		
All 3 means are significantly different from one another		

Table 7: *LSD all pairwise comparison test of CS for time.*

Time	Mean	Homogenous group
3	3.5600	A
1	3.0833	B
2	2.6467	C
Alpha 0.05	Standard error for comparison 0.0528	
Critical T value	Critical value for comparison 0.1466	
Error term used: rep*time,4DF		
All 3 means are significantly different from one another		

Table 8: *Proximate Composition of (Pennisetum purpureum) at three growth stages (days) on dry matter basis.*

Factors	Twenty one	Forty five	Sixty
Protein content (%)	6.91	6.83	6.40
Crude fiber (%)	31.41	32.10	33.40
Ash (%)	9.20	9.10	10.20
Ether extract (%)	1.62	1.58	1.44
Nitrogen free extract (%)	49.10	48.60	47.38
Total digestible nutrients (%)	59.30	59.20	57.54

The biomass production is determined by the morphological, physiological and biochemical processes happening in any plant due to change in the atmospheric in a soil environment (Zahid *et al*., 2002). The research study was conducted in the RRI, NARC, Islamabad. The result indicated that showed their increasing pattern in dry matter yield. The more the time interval and the more time in the soil, the more and improved yield was achieved. The increasing

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trend in the dry matter yield of Mott grass highlighted the nutritional capability of the soil of Barani areas of this region. Table 1 reflects the elevated dry matter of the grass specie as its ability to hold about 3882 kg/ha of weight after 60 days of interval. As the yield was reportedly influenced by the uptake of essential minerals in the soil, this indicate that the soil of this region can support the productivity of elephant grass (*Pennisetum purpureum*) irrespective of their other nutritional parameters.

Comparatively similar research was carried out on the capacity of Mott grass was analyzed by application of enhanced fertilizers. Mott grass specie was analyzed for it dry matter yield and crude protein factor against the application of nitrogen fertilizer. The results of the study deduced that as the interval time of the grass is increased the dry matter of is enhanced and the yield is improved (Zahid *et al*., 2002).

Talking about the carrying capacity, it is the quantitative approach that measure the limit of organism in a particular area based on the environmental condition and resources available. Four common factors like food availability, enough supply of water, space to live in and good environmental condition effects the capacity of an area. The study was done to highlight the Carrying capacity of the Mott grass based on to how much Animal unit per hectare the specie can support. After the 60 days harvesting interval the Mott grass can support up to 7.0 AU/ha showing the maximum carrying capacity (Figure 3). This indicates that Mott grass can provide a better resource to the organisms in limited time and can prove to be available during stress condition.

Similar study was carried out by (Sultana, 2000) that highlighted the carrying capacity of the pasture in the Pothwar region where Mott grass was examined for dry matter, carrying capacity and crude protein comparative to other grass like blue panic and sesbania. Elephant or Mott grass were significantly more productive yielding 25-30 tons (22679-27215 kg) dry matter per hectare compared to others, similarly a highly significant difference was seen in carrying capacity of the grasses as Mott grass was capable of supporting about 50 AU/ha. The study concluded that elephant or Mott grass are highly productive than other grass species for providing better and instant forage production (Sultana, 2000).

Energy crops have the capacity to sequester the amount of carbon back into the earth almost equal to the C emitted during burning. Moreover, the potential of such crop is determined by C addition, its capacity of storage and the stability in the soil (Mclaughlin *et al*., 2002). The study carried out at NARC also highlights the carbon up taking capacity of Mott grass. Figure 4 highlights that with the increase in harvesting time of the grass there is more chances to capture more amount of atmospheric carbon into the soil. After the decline of carbon content after 45 days the carbon storage rise up to 3175.144 kg/ha after harvest time of 60 days. Similar study was carried out in which the proximate and structural analysis was done. The experiment analyzed the amount of Napier grass stem, leaf to check the carbon content of the grass using thermo-gravimetric analyzer. The result indicated that the Napier grass has high carbon content and high heating value.

Chemical analysis or proximate analysis determines the compound in the mixture of substances. Proximate analysis highlights the protein and ash content of the compound and is increasingly important in knowing the nutritional value of the compound. Under the study carried out, Mott grass was evaluated for five factors i.e. protein content, crude fiber, ash content, ether extract, nitrogen free extract and total digestible nutrients. According to the data observed the protein content of the plant was reduced to 6.91 to 6.40 in the duration from 21 days to 60 days. Ash and crude fiber content of the Mott grass was increased after 60 days of harvesting with the inverse relation to it nutritional value, which reduced in the desired period (Table 8).

A similar study carried out by (Aganga *et al*., 2005) highlighted that young Napier grass harvested is high in digestible nutrient if cut at 50 cm of height but when the maturity of the plan is increased the yield increased but quality of the plant is reduced giving an inverse relation to the production.

Conclusions and Recommendations

The study carbon sequestration and biomass assessment of Mott grass (*Pennisetum purpureum*) in three growth stages in barani areas of Pothwar Pakistan was carried out in the area of NARC, Islamabad. The experimental plot was designed over an area of one acre. Row to row distance was kept at ninety centimeter and plant to plant distance was retained at sixty cm grown with the stumps. The Mott grass was analyzed for dry matter weight, carrying capacity and carbon stock at three growth stages. Moreover, the plant was analyzed for proximate evaluation by checking the crude protein, ether extraction, ash estimation, and crude fiber content. By assessing the data obtained through experimentation and ANOVA interpretation significant results $P< 0.05$ were obtained that Mott grass can prove to be effective in providing high yield biomass in limited time and also have the capacity to support organisms and provide good quality fodder to animals round the year on sustainable basis. Moreover, the grass specie can help reducing the pollution burden on environment by sequestering the emitted carbon from the ecosystem.

It is suggested for future scientists and connected divisions to additionally examine and evaluate the dietary benefit alongside these factor. It will additionally open its way into the agricultural system of Pakistan to give quality forage consistently.

Novelty Statement

Carbon sequestration in grasslands is that implementation of practices to sequester carbon often lead to increased production and greater economic returns. Forage removal that disturb the system and prompt carbon losses usually to enhance forage utilization. Reducing the amount of carbon inputs removed, or increasing production, carbon inputs or below-ground allocation, could all lead to increasing soil carbon stocks. Sustainable grazing management can thus increase carbon inputs and carbon stocks without necessarily reducing forage production. Grazing management can also be used to restore productive forage species, further augmenting carbon inputs and soil carbon stocks.

Author's Contribution

Sheza shehzadi: Writing, management of the article and lab work.
Mohammad Umar Farooq: Conceive the idea, research support at every step.
Rukhsana Kausar: Editing help during the writing.
Ijaz Ali: Support in data analysis and lab work.
Arshad Ullah: Support in editing.
Maqbool Shahbaz: Technical support at every step.

Conflict of interest

The authors have declared no conflict of interest

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