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Study of Asexual Propagation of Moringa (Moringa oleifera) on Biomass Yield, Nutritional Composition and In-sacco DM Degradability

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Authors' contributions

This work was carried out in collaboration between all authors. Author KSH designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MKB managed the literature searches, analyses of the study performed the spectroscopy analysis and author KSH managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Aims: The aims of this study were undertaken with the objectives to determine asexual propagation of moringa plant using branch cuttings of different maturities indicated by the cutting diameter of A) 3-5 cm, B) 6-8 cm or C) 10-12 cm on biomass yield, nutritional composition and In-sacco DM degradability.

Study Design: The design of the study is completely randomized design (CRD).

Place and Duration of Study: The agronomical trial was conducted at Pachutia fodder plot of the Bangladesh Livestock Research Institute (BLRI) from June, 2014 to May, 2015.

Methodology: Twelve (12) plots each area with 800 x100 cm² were prepared with basal dose of fertilizer (kg) of cow dung, urea, Tipple Super Phosphate(TSP) and Murat of Potash(MP)

(27000:90:30:15), and branch cuts were planted at a space of 30x30 cm randomly replicating each type of cut into four (4) different plots. The experimental data on survivability (%), no. of prunes per cut, yield of different botanical fractions of moringa foliage, chemical composition and In-sacco DM degradability were recorded.

Results: The survivability (72.4%) or the number of prunes/cut (4.55) of 10-12 cm branch cut were significantly ($P = .001$) higher than that of the branch cuts with 3-5 cm (5.62% & 1.92, respectively) or 6-8 cm diameter (8.41% & 2.70, respectively). The fresh and dry matter yield (ton ha⁻¹ yr⁻¹) of total foliage, stem and leaf were significantly $(P=0.001)$ higher at 10-12 cm branch diameter followed by 6-8 cm and 3-5 cm branch diameters, respectively. Effect of leaf to stem ratio was not significant among the treatments. Crude Protein (CP) content of all fraction of different cuts did not vary (P=0.193) significantly. The acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) of total foliage was lower in 10-12 cm branch diameter compared to other diameters. It was observed that, the ADF: CP ratio (2.0 to 2.5) was appropriate for effective DM degradability (%) which was 53.0-54.0%.

Conclusion: Both yields and chemical composition of moringa foliage suggested that 10-12 cm branch diameter used for asexual propagation and could be a potential protein source for ruminants livestock.

Keywords: Branch diameter; propagation; heat index temperature; biomass and nutritional evaluation; fodder production.

1. INTRODUCTION

The acute shortage of feeds and fodders both in terms of quality and quantity is one of the major barriers to feeding farm animals in Bangladesh. The country quantitively requires 49.0 million tons DM of roughage and 24.0 million tons of concentrate in a year [1] and meets only 56.4% and 20.0%, respectively of their requirements. In addition to this deficiency in feed biomass, access of high yielding animals to diet especially of high metabolizability and quality protein restricts exploitation of their genetic production potentials of milk and meat and even impact product, quality and climate [2-4]. Exploration of options for increasing production and supply of good quality fodders is of prime importance. Moringa (Moringa oleifera) may be one of the best quality plant fodders to support increased farm animal production and productivity [5-7]. It is a fast growing native tree of Bangladesh and grows almost in every types of soil. Moringa leaves are rich in crude protein (CP),vitamins and essential minerals like iron, potassium, calcium [8,3] and feeding moringa leaves increased live weight and milk production by 32% and 65%, respectively [9,10-12,2]. Moringa foliage diet is 3.29 times efficient than the Napier diet is converting feed into live weight gain of growing male goat [6]. It may support commercial protein meal feed production in the country, and if produced and processed sustainably and economically and may reduce import of premixed feed [1]. Farmers produce moringa conventionally at their home stead's for

producing drumsticks without any use of foliage as livestock feed. They use asexual propagation for multiplication of the plant. It is not familiar to them as a plant fodder and its cultivation system as a fodder crop is totally unknown. However, conventional asexual propagation system may be used for production of moringa as a fodder crop and determinations of maturity aerial vegetative parts (branches) and its impacts on pruning efficiency, biomass yield and chemical composition are also important. Moreover, temperature and humidity are important climatic factor that affect biomass production [13,14]. Considering the above factors, the present work was undertaken to determine the optimum maturity of aerial vegetative parts of moringa plant on plant fodder production and their quality.

2. MATERIALS AND METHODS

2.1 Location and Agro-climate of the Experimental Site

The agronomical trial was conducted at Pachutia fodder plot of the Bangladesh Livestock Research Institute (BLRI) from June, 2014 to May, 2015. The station was located at 23°42'0'' N, 90°22'30'' E at the altitude of 4 m above the sea level. The soil classification belongs to the Madhupur Tract (Agro-Ecological Zone 28) of Bangladesh; Red- Brown Terrace strong acidic (pH 4.5-5.7) soil with very little $(<1.5\%)$ organic matter. The land area was composed of alluvium soil of the Pleistocene period. The soils of this tract had clayey texture.

2.2 Preparation of Experimental Plot

The different maturities of moringa branch were collected from different place of research center of BLRI and planted by the different branch diameter of A) 3-5 cm, B) 6-8 cm or C) 10-12 cm in the month of March with a temperature ranges of 29 - 40ºC and humidity ranges of 41 to 50%. A basal dose of fertilizer like cow dung, urea, Tipple Super Phosphate (TSP) and Murat of Potash (MP) was applied at the ratio of 2700:90:30:15 kg per hector. Irrigation; weeding and other agronomical practices were common.

2.3 Experimental Layout Design and Treatment

The total area of experimental plot was 1270000 cm^2 and planted with 720 plants. Twelve (12) plots with 800 $x100$ cm² were prepared with a total of 60 plants per plot. The entire sapling of branch cutting were transplanted at a distance of 30x30 cm randomly replicated each type of branch diameter into four (4) different plots.

2.4 Harvesting Procedure and Sample Collection

The re-growth's foliage was harvested using a long knife at 60 days interval to maintain uniform height above the ground during the experimental period. The total fresh matter was harvested from each plot, weighed, and registered to estimate fresh matter yield. Fresh yield was considered as total foliage. The total fresh biomass yield from each plot was separated into two fractions: leaf and stem. Leaves and stems were weighed to determine fresh yield of leaves and stems and ratio of leaves and stem of total foliage at DM basis. The chemical composition in terms of fresh dry mater (DM) and crude protein (CP) was analyzed in the animal nutrition laboratory of BLRI.

2.5 DM yield of Total Foliage Leaves and Stem

Samples of plant fractions (total foliage, leaves and stems) were chopped into short length (3 to 5 cm) and dried in air-forced oven at 100°C for 24 h for DM determination. Fresh yield was converted to DM yield plot- 1 ha 1 according to the equation:

DM yield plot⁻¹ = Weight of fresh material \times DM (%).

2.6 Chemical Analysis

Ash determination was done at 550° C for 8 hours. Total nitrogen (N) was determined by the semi-micro Kjeldahl procedure and CP calculated from N content (CP=Nx6.25) according to the official methods of AOAC [15]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the procedure proposed by Van Soest et al. [16].

2.7 Ruminal Degradation Kinetics of Moringa Foliage

Three fistulae local growing bulls weighed in average 225 kg were used to determine DM degradability of moringa foliage. Animals were fed a basal diet of concentrate feed mixture at 2.25 kg/head/ day. Tested forages were oven dried; finally ground and two grams of each were weighed in triplets of moringa foliage for each incubation time. Nylon bags technique was applied to determine degradability of the moringa foliage according to Mehrez and Orskov [17] using polyethylene bags (7x16 cm) with pore size 45 µ m. Moringa foliage samples were incubated in the rumen of the fistulae local growing bulls for 8, 16, 24, 48 and 72 hrs. All bags were rinsed in tap water for 15 minutes until the water become clear, squeezed gently, oven dried and weighed to determine zero-time washing loss (a). Degradation kinetics of DM were estimated by fitting the disappearance values to the equation $P=$ a+ b (1-e- c ^t) as proposed by Orskov and McDonald [18] where P represents the disappearance after time t. Least-squares estimated of soluble fractions are defined as the rapidly degradable fraction (A), slowly degradable fraction (B) and the rate of degradation (C). The effective degradability (ED) for tested moringa foliage was estimated from the equation of McDonald [19] where: ED= a+bc/(c+k), k is the out flow rate.

2.8 Statistical Analysis

The experimental data on the rate of survival (%) and death (%), the number of prunes per cut, fresh yield and DM yield of different botanical fractions of moringa foliage, proximate analysis, fiber content and digestibility In-sacco were recorded. The data was analyzed in an ANOVA of completely randomized design (CRD) using SPSS-17.0 statistical software program.

3. RESULTS

The effects of the cutting diameter on survival rate (%), death rate (%), number of prunes, Arial prunes (%) and sub- soil prune (%) are given in Table 1. It shows that about 72.4% branch cuts of 10-12 cm survived with an average of 4.55 prunes/cut. Of them 42.4% pruned aerially and the rest 57.36% pruned from the sub-soil part.

Their survivability (72.4%) or the number of prunes/cut (4.55) was significantly $(P = .001)$ higher than that of the branch cuts with 3-5 cm $(5.62\% \& 1.92,$ respectively) or 6-8 cm diameter $(8.41\% \& 2.70,$ respectively), and the (8.41% & 2.70, respectively), and the performance of the latter two cuts of 10-12 cm diameter had a comparatively higher number of prunes from sub-soil part (57.36%) compare to the aerial part (42.63%). It had significantly (P $=$.013) lower aerial pruning and higher (P = 034) sub-soil pruning. An average trend is shown by the cuts 3-5 cm or 6-8 cm diameter, and they had 52.42% and 67.19% aerial pruning respectively; and 47.58% and 34.92% pruning respectively. Their aerial pruning performance was significantly $(P = 013)$ higher and sub-soil pruning was significantly $(P = .034)$ lower than that of other cuts. Higher maturity of branch cuts with mature cells indicated by the higher diameter of branch cuts favored a high survival rate, more prunes/cuts and an average percent of sub-soil pruning.

3.1 Biomass Yield

The effects of different cutting diameter on fresh and dry matter yield of total foliage, leaf and stem fractions of moringa (Moringa oleifera) and leaf stem ratio are shown in Table 2. The fresh yield of total foliage, leaf and stem fractions per cut or per year increased significantly ($P = .001$) as the cutting diameter prolonged from 3-5 cm to 10-12 cm. The branch diameter of 10-12 cm produced highest fresh biomass; it was 48.14 ton per hector per year on average. The 6-8 cm cutting diameter was the second largest biomass producer (17.75 ton/ha/year) and 3-5 cm diameter ranked the third position (15.32 ton/ha/year) respectively. The total dry matter (DM) of all parts of moringa plant was substantially greater $(P = 0.001)$ for the largest branch diameter (10-12 cm) than for the other two diameter. It also shows that the total amount of biomass production was three times more in 10-12 cm branch diameter than that of 3-5 cm branch diameter. The 3-5 cm branch diameter produced lowest dry matter 3.01 ton per hector per year. The biomass production rose sharply from 3-5 cm to 10-12 cm cutting diameter, it was 9.8 ton per hectare per year. The effect of leaf to stem ratio was not significant among the different cutting diameter.

3.2 Nutrient Composition

The effects of three cutting diameter on chemical composition of different botanical fractions of moringa are presented in Table 3. The DM of total foliage, leaf and stem were not significantly different among the treatments. The highest DM content of whole foliage was obtained at 10-12 cm cutting diameter whereas the lowest DM content of whole foliage was recorded from 6-8 cm cutting diameter. The DM content of total foliage, stem and leaf ranged from 183.12 to 197.22, 156.70 to 163.42 and 215.85 to 241.92 g kg⁻¹, respectively. The CP content of total foliage, leaf and stem were not significantly affected among the different cutting diameter. It was observed that, 10-12 cm cutting diameter of total foliage obtained highest CP (213.95 g kg⁻¹) compared to two diameters. There is no significant different of ADF content of total foliage, stem and leaf among the three treatments. Moreover, the ADF content of all fractions did not changes over the different cutting diameter. The NDF content of total foliage, leaf and stem were not significant $(P = .390)$ effect on three different cutting diameter. The mean of NDF content was highest in stem fraction (724.70 g kg^{-1}) associated to the total foliage $(426.12 \text{ g kg}^{-1})$ and leaf (351.35 g) kg^{-1}) respectively. Increasing the cutting diameter did not have any significant influence on ether extract (Crude fat) content of all plant fractions. The mean of ether extract content was higher in total foliage (277.85 g kg^{-1}) compared to the stem fraction (86.50 g kg^{-1}). The ADL content of total foliage, stem and leaf fraction were not significant different among the three cutting diameters. The ADL value of leaf was unchanged over the different diameter. The lignin content of stem was more distinct compared to total foliage.

3.3 Degradation Kinetics

Estimates of ruminal degradation contents (a, b and c) fitted with rate of DM disappearance of tested moringa foliage is presented in Table 4. The results illustrate that "a" washing loss of fraction 10- 12 cm was significantly $(P = 035)$ higher (23) than that of 3-5 cm (20.9) 0r 6-8 cm (21.53) respectively. Branch diameter of 10-12 cm of degradable fraction "b" rate of degradation

Parameters	Cutting diameter (cm)			Significance	
	$3 - 5$	$6 - 8$	$10 - 12$	Overall SE	Level
Survival rate (%)	5.62° ±0.54	$8.41^{b} + 0.46$	$72.44^a \pm 5.35$	1.79	$***$
Death rate (%)	$94.37^b \pm 0.54$	$91.58^b \pm 0.46$	27.55° ±5.35	1.79	$***$
Number prunes	1.92° ±0.13	2.70° ±0.35	$4.55^a \pm 0.27$	0.15	$***$
Aerial prune %	$52.42^b \pm 5.35$	67.19° ±4.88	42.63° ±3.01	2.61	\star
Sub-soil prune %	$47.58^b \pm 5.37$	34.92° ±6.10	57.36° ±3.01	3.39	\star

Table 1. Effect of cutting diameter on agronomical performances of moringa (Moriga oleifera)

Figures with different superscript in the same row differ significantly at ***=P < .001 or $* = P < 0.01$ or $E^* = P < .05$ level

Figures with different superscript in the same row differ significantly at *** $= P < .001$ or NS= Non-significant

"c" and effective degradability "ED" of DM were lower ($P = 145$) than 6-8 cm branch diameter, meanwhile 3-5 cm branch diameter was associated with improving all degradable fractions than for 10-12 cm branch diameter but lower than 6-8 cm branch diameter.

The relationship between the Heat Index Temperature (ºF) and the dry matter (DM) yield of different branch diameter shown in Fig. 1. It was observed that the increase of branch diameter is higher than rate of increase of dry matter yield (ton/ha) (1.98 VS 0.77 VS 0.30) for unit increase of Heat Index Temperature (ºF). The fig. also shown that, Heat Index Temperature (ºF) directly impacts on biomass production and strong liner relationship among the treatment. It was identified that, the range of Heat Index temperature 25 to 32º F is suitable for higher biomass production with 10-12 cm cutting diameter.

The relationship between ADF: CP ratio and effective DM degradability in the rumen of three different branch diameter is shown in Fig. 2. It shows that, effective DM degradability (%) was decreased with the increase of branch diameter. It was observed that, the ranges of ADF: CP ratio 2.0 to 2.5 is appropriate for effective DM degradability (%) that was 53.0-54.0% and higher DM production (ton/ha) that was indicated by 10-12 cm cutting diameter.

4. DISCUSSION

The result shows that, the survival rate (%) and number of prunes were increased significantly (P =.001) with the increased of branch diameter. The highest survival rate (%) of moringa was obtained from 10-12 cm cutting diameter (72.44%) whereas lowest in 3-5 cm branch diameter (5.62%), respectively. This result is partially comparable [20] at 14-16 mm and 17-20 mm branch diameter with the survival rate (%)

Huque et al.; IJPSS, 11(4): 1-11, 2016; Article no.IJPSS.26103

ranged from 5 to 15 and 5 to 35%, respectively. Actually, when the moringa are grown from higher diameter of stem cutting, adventitious root system are found. But the moringa have deep tap root system when they are grown from seed [3,21-22]. It is noted that, amplified survival rate (%) and number of prunes for increasing the cutting diameter of 3-5 cm, 6-8 cm and 10-12 cm, respectively.

The result also shows that, both fresh and dry matter yield (ton ha⁻¹ cut)⁻¹ or (ton ha⁻¹ yr⁻¹) were linearly $(P = 0.001)$ increased with the increased of branch diameter. The highest DM yield (ton ha^{-1} yr⁻¹) of all fractions of moringa was obtained from 10-12 cm branch diameter due to higher fresh biomass produced of all fractions (Table 2). In the present study, the annual DM productions of

Figures with different superscript in the same row differ significantly at $*= P < .05$ or NS= Non-significant. DM= Dry matter; CP= Crude protein; ADF= Acid detergent fiber; NDF= Neutral detergent fiber; EE= Ether extract; ADL= Acid detergent lignin

Figures with different superscript in the same row differ significantly at $*= P < .05$ level; NS= Non- significant

total foliage were 3.01, 3.56 and 9.80 ton ha⁻¹ yr^{-1} at 3-5 cm, 6-8 cm and 10-12 cm branch diameter, respectively. The results are partially supported to Palada et al. [23] produces higher dry matter between 4.2 and 8.3 ton ha^{-1} yr⁻¹ depending on fertilizer, accession, season and ecological zone. Yield obtained in the present study were lowest than those reported [24] who exhibited that DM yield of total foliage was 99 ton ha $^{-1}$ yr⁻¹ if 1 million plants per hectare is cultivated and harvested nine time per year and this practice may continue even up to 4 years. Goss also reported that similar findings [25].

The leaf stem ratio in the present study was similar at 3-5 cm, 6-8 cm and 10-12 cm branch diameter but it was non-significantly slightly higher in 10-12 cm cutting diameter. This finding is similar to Sultana et al. [26] that was no significant different among the treatments. The leaf and stem production in tree forage is more closely related to temperature and particularly to rainfall which influence forage quality by altering leaf stem ratio [27].

The dry matter (DM) content of all fractions of moringa was not significantly different among the three different treatments. The DM value obtained at 3-5 cm 6-8 cm and 10-12 cm branch diameter in the present study were similar to those obtained by Reyes-Sanchez et al. [28] and Mendieta et al. [29].

The effects of branch diameter on CP content of moringa foliage, stem and leaf were not significant. In the present investigation, the CP content of moringa was within the ranges of 193- 264 g kg⁻¹ DM reported for moringa by other workers [26,30-36]. The CP ranges from 299.05 to 308.62 q kg⁻¹ DM of moringa leaves obtained in this study which was appeared to be lower than that reported by Soliva [37] (320 g kg⁻¹ DM). The CP content of moringa stem in comparatable to mature forages such as Napier grass (Pennisetum purpureum) (79.9 to 109.0 g kg-1 DM) or guinea grass (91.7 g kg-1 DM) as informed by Ansah et al. [38] or Fadiyium et al. [39]. The mean CP value of moringa foliage (209.96 g kg-1 DM) and leaf (294.98 g kg-1 DM) was considerably higher than the mean CP content of forage legumes (170.0 g kg-1 DM) and grasses (115.0 g kg-1 DM) as reported by Minson [40].

Branch diameter had no significant effect on ADF, NDF and ADL content of all fraction of moringa during the year (Table 3). The same trend was reported by Al-Masri [34]. The effect of branch diameter on fiber compounds was more in total foliage and stem fractions compared to leaf indicating that leaves have less structural materials than the stem. The young stems are generally of high quality but the quality decrease faster than in leaves, because epidermis and fibrous cells changes secondary cellular wall and lignin content increase with increased age of the plant. The ADF and NDF content of total foliage obtained in this study for the different branch diameter within the ranges of 414.70 to 422.6 g kg⁻¹ DM and 422.6 to 426.1 g kg⁻¹DM reported by other researchers (Makkar and Becker [30] Foidle et al. [32] Aregheore [33] Al-Masri [34]) for ADL and NDF. The range of ADF values $(215.07$ to 217.75 g kg⁻¹ DM) of leaves in the present study is not agreement with Nouala et al. [41] and Manh et al. [35] whose values were 160.0 and 170.0 g kg-1 DM, respectively.

Fig. 1. Heat index temperature (F) impact on Moringa production

Fig. 2. Relation of ADF: CP ratio with effective DM degradability (%)

The range of NDF values (702.75to 724.70 g kg-1 DM) of stem fraction of moringa obtained in this study is higher than the findings of Buxton and False [27] (657.6 to 683.1 g kg 3 DM) at different densities. The high NDF content in stem fraction is related to anatomical structure of the moringa plant by which fiber exists mostly in the stem for support by Van Soest [16].

ADF and NDF contents in total foliage and leaf were 414.70 and 420.90 g kg^{-1} DM and 215.08 and 338.65 g kg^{-1} DM, respectively, at 10-12 cm branch diameter. These values are comparable to recommended level for ruminant (25-35%) for NDF and up to 23% for ADF as informed by Norton et al. [42] and Lu et al. [43]. It can be recommended for ruminants' livestock.

The values of ADL for leaves (65.73 g kg $^{-1}$ DM) in the present study were lower compared to Buxton and False $[27]$ (82.0 g kg⁻¹ DM). The low fiber content in moringa leaf makes it potential protein source to poultry and other monogastric animals. A comparatively higher mean ADL value $(232.1 \text{ g kg}^{-1} \text{ DM})$ in stem was found in the present study than that reported by Mendieta-Araica et al. [29] (111.0 g kg-1 DM). Factors such as differences in agro-climatic conditions, soil type and fertilization age of trees stage of maturity of the leaves different parts of the plant sampled (leaves, twigs, branch, and stem) could have contributed to some of the differences between reported values.

Effective dry matter (DM) degradability was not significantly ($P = 145$) different among the three branch diameter of moringa. In the present investigation, IVDMD of moringa was within the

ranges of $517.9 - 542.2$ g kg⁻¹. The result of effective DM degradability of moringa foliage in this study is lower than the findings (648-790 g kg^{-1}) of other researcher [30-35]. Moringa foliage can also partially replace cottonseed meal or other proteinaceous forage of goat reported by Aregheore [31] and Asaolu et al. [44].

5. CONCLUSION

From the results it may be concluded that cuttings with 10-12 cm branch diameters can be used for asexual propagation for Moringa as fodder production in Bangladesh. Evaluating the nutritional composition of moringa (Moringa oleifera) it could be a suitable source of protein for livestock feeding in the country. Therefore, further investigation is needed to develop cost effective agronomical practices for the propagation of Moringa as fodder crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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