Forage Res., **46** (4) : pp. 356-362 (2021) **http://forageresearch.in**

EFFECT OF PRE-TREATMENTS ON QUALITY OF MAIZE (*ZEA MAYS* L.) STOVER

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(Received : 10 February 2021; Accepted : 30 March 2021)

SUMMARY

To improve quality and digestibility of maize stover for meeting the nutritional requirements of livestock, it was subjected to different pre-treatments. Treatment of maize stover with sodium chloride (2%) resulted improvement in CP content (18.43%) and *in vitro* dry matter digestibility (16.80%). The decrease in ADF (7.35%) and cellulose content (3.40%) was also observed after the treatment. Pre-treatment of stover with calcium hydroxide revealed increase in total carbohydrate and ash content of stover. The calcium hydroxide treatment was also effective in lowering the NDF (8.80%) and ADL content (55.30%) of stover. Treatment with ammonia resulted significant reduction in silica (52.63%), total phenolic (67.70%), simple phenolic (66.90%) and total tannins content (68.60%). Maize stover inoculated with *Pseudomonas fluorescens* revealed increase in CP content (49.10%) and decrease in ADF (5.40%), cellulose (1.60%) and simple phenolic (59.40%) contents. Treatment with *Trichoderma viride* resulted 27.70% increase in total carbohydrate content of stover. Highest reduction in silica content (47.40%) was recorded with *Saccharomyces cerevisae* treatment. Inoculation of *Trichoderma harzianum* lowered the NDF (5.79%), hemicellulose (16.50%), total phenols (34.40%) and total tannins (18.90%) content of stover. This treatment was also effective in improving stover digestibility up to 17.84%. The study indicated that treatment of maize stover either with alkali or inoculation with *Pseudomonas fluorescens* and *Trichoderma harzianum* are effective for improving nutritional quality of maize stover.

Key words : Maize stover, nutritive quality, delignification, digestibility, chemical and biological treatments

Crops and livestock are the mainstream enterprises intrinsically linked to each other in mixed farming system. India is an agrarian country where both components have considerable share in national GDP. Livestock production shares about 4.11% of total GDP and 25.60% of total Agricultural GDP (Anonymous, 2012). Despite holding largest population of the livestock and making significant contribution in sustaining rural livelihoods and national economy, the livestock productivity in the country is comparatively lower. The irregularity in fodder availability is one of the prominent factors for low livestock productivity in the country. Further, this situation has been aggravated because of teeming human population which has put extreme pressure on agricultural lands to produce more and converted farmlands into commercial landscapes (Ayyadurai *et al.,* 2013). Consequently, the cultivated area under the forage crops is continuously declining. Presently, the country is facing a net deficit of 35.60% green fodder, 10.95% dry crop residues and 44% concentrate feeds (IGFRI Vision-2050). The percentage proportion of fodder unavailability is expected to increase continuously and to reach 1012 million tonnes of green fodder and 631 million tonnes of dry forage by the year 2050 (Ghosh *et al.,* 2016). During the inadequacy of green fodder, livestock mainly depends on fibrous feeds such as straws, husks and stover which have low nutritive value and low digestibility (Asmare, 2020). These issues coupled with increasing demand for livestock products due to intense urbanization and expanding human population necessitate the research on better utilization of crop residues for improving livestock production (Mahesh and Mohini, 2014; Katoch *et al.,* 2017).

Maize (*Zea mays*) is one of the most versatile crops known as “Queen” of cereals crops. India holds second position in maize production among other maize growing Asian countries, while sixth position in the world. Maize possesses the characteristics of an ideal food-feed crop (Katoch and Kumar, 2014). The rapid increase in both human and livestock population, along with a restricted land base and an increased demand for livestock products has further increased the demand of maize as a food-feed crop. Being a dual purpose crop, maize holds great potential to improve socioeconomic status of rural families by ensuring food as well as feed security (Katoch *et al.,* 2009; Arya *et al*., 2020). Maize stover, one of the major crop residues, is characterized by multi-source, wide distribution, high abundance, low cost and less competing usage (Apoorva *et al.,* 2019). It is primarily used for livestock feeding often through *in situ* stubble grazing and/or as *ex situ* forage. The value of maize stover increases during inadequacy of green fodder. Despite of such valuable characteristics, maize stover is not used as potential livestock feed due to inherently low voluntary intake and low digestibility (Casperson *et al.,* 2018).

High fiber content and presence of complex structures (ligno-cellulosic complex, tannin-protein complex) and silica in cell wall represent a barrier that prevents the growth of ruminal microflora and enzymatic hydrolysis of wall polysaccharides. These factors are collectively responsible for low quality and digestibility of stover.

In India, about 500 million tonnes of crop residues are produced annually, of which about 15% is stover (Devi *et al.,* 2017; Bhuvaneshwari *et al.,* 2019) which could be utilized for livestock feeding. Looking at the vast gap between the demand and supply of livestock feeds, the utilization of maize stover as a potential livestock feed is necessary to meet the feed inventory needs. Keeping this under consideration, the present investigation was undertaken to study the effect of different chemical and biological treatments on nutritional quality and digestibility of maize stover.

MATERIALS AND METHODS

Sample preparation : The stover samples of maize (African tall) were collected during the months of October and November from the research farm of CSKHPKV, Palampur, Himachal Pradesh, India. The samples were dried at 50°C to the constant weight and ground in a Willey mill (1 mm sieve) and preserved in air tight polythene bags for further analysis.

Pre-treatment of samples

Chemical treatment : In this treatment, 5gm stover was mixed with 50ml of sodium hydroxide (2%) and calcium hydroxide (2%) solutions for 30 mins and 1 h, respectively. For acid hydrolysis, stover samples were treated with 50 ml of 1.50% sulphuric acid for 3 hrs at 100°C. The experimental material was treated with sodium chloride (2%) and urea (4.50%) for overnight and 21 days, respectively at room temperature. Treatment of stover with ammonium hydroxide (10%) was carried out at room temperature for 24 hrs. Following aqueous ammonia steeping, the delignified sample was further hydrolyzed with 0.30M HCl at 100°C for 1 hr. After each treatment, the samples were washed, dried and stored at ambient temperature for further analysis.

Biological treatment : The effect of *Trichoderma harzianum*, *Trichoderma viride*, *Saccharomyces cerevisae*, and *Pseudomonas fluorescens* treatments on the quality and digestibility of maize stover was investigated. The mother culture of these microorganisms was obtained from the Department of Plant Pathology, CSKHPKV, Palampur which were subsequently sub-cultured. *T. harzianum, T. viride* and *S. cerevisiae* were sub-cultured on potato dextrose broth, while *P. fluorescens* was sub-cultured using Kings B broth. The pasteurized stover samples were inoculated and incubated for 7 days. After 7 days, inoculated samples were oven dried, stored in a polythene bags for further nutritional analysis.

Nutritional analysis

Crude protein (CP) and ash contents were estimated according to the method of AOAC (2000). The cell wall constituents *i.e.,* Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL), hemicellulose (HC) and cellulose (CL) were determined according to the method of Goering and Van Soest (1975). The samples were also analyzed for total carbohydrates and reducing sugars content by following the method of Hedge and Hofreiter (1962) and Miller (1972), respectively. Total phenols (TP), simple phenols (SP) and total tannins (TT) were estimated by the method of Makkar (2003). The silica content was determined by following the method of Van Soest and Wine (1968). The *in vitro* dry matter digestibility was estimated by following two stage digestion method of Tilley and Terry (1963). The significant differences in results were detected by analysis of variance (One-Way ANOVA) (Panse and Sukhatme, 1984). A probability of ? 0.05 and 0.01 was selected as criterion for statistically significant difference.

RESULTS AND DISCUSSION

Effect of chemical and biological treatments nutritive quality of maize stover

The variations in the level of different nutritional constituents of maize stover after chemical and biological treatments have been presented in Table 1 & 2.

Crude protein and carbohydrate content : Crude protein is one of the absolute dietary nutrients to livestock as it supports the growth of ruminal microflora and consequently increases the nutrient assimilation efficiency of animals (Katoch *et al.,* 2018). The treatment with sodium chloride resulted highest improvement in CP content (18.43%) of stover, followed by diluted sulphuric acid (16.30%), urea (10.20%) and aqueous ammonia (6.18%) treatments (Table-1). Chea *et al.* (2015) reported 5.90% increase in CP content of salt treated maize stover. It has also been reported that urea treatment increases CP content from 6% to the extent of 131% in maize stover (Elias and Fulpagare, 2015; Abera *et al.,* 2018; Ma *et al.,* 2020). Treatment with urea and ammonia increases non-protein nitrogen content in feeds which destabilizes protein complexes with anti-nutrients thereby

TABLE 1
Effect of chemical treatments on quality attributes of maize stover

	Control	Urea (4.50%)	NH_4OH (10%)	NaOH (2%)	$Ca(OH)_2$ (2%)	NaCl (2%)	Dil. H_2SO_4 (1.5%)	SE	CD (5%)	CD (1%)
CP (%)	8.57	9.45*	9.10*	7.35^{b}	4.55^{bd}	10.15^{ac}	9.97^{a}	0.51	1.11	1.52
TC (%)	13.04	8.01^{bd}	8.05^{bd}	13.81*	20.59^{ac}	11.79*	13.53*	0.85	1.84	2.53
RS (%)	6.24	6.71	7.02	7.68	7.70	6.16	7.85	1.03	NS	NS
NDF (%)	69.00	67.00*	66.70*	63.30b	62.90^{b}	65.20^{b}	67.40*	1.54	3.34	NS
ADF (%)	46.20	43.10	43.50	42.80	43.30	44.50	46.00	1.58	NS	NS
IVDMD(%)	59.40	68.20^{ac}	69.00^{ac}	69.40^{ac}	62.40^{ac}	62.20^{ac}	65.00^{ac}	0.20	0.42	0.60
ADL (%)	4.70	2.50^{bd}	2.90^{bd}	2.70^{bd}	2.10^{bd}	3.40^{bd}	4.00^{bd}	0.11	0.24	0.33
HC (%)	22.80	23.90	23.20	20.50	19.60	20.70*	21.40	1.97	NS	NS
Cel (%)	41.50	40.60	40.60	40.10	41.20	41.10	42.00	1.60	NS	NS
Ash (%)	3.80	3.40*	4.80*	4.90*	5.45^{a}	5.00*	3.53*	0.67	1.43	NS
Silica (%)	1.90	2.00*	0.90^{bd}	1.70*	1.70*	2.27^{a}	1.50^{bd}	0.13	0.28	0.39
TP (mg/g)	12.58	8.00^{bd}	4.06^{bd}	6.06^{bd}	5.61^{bd}	6.96^{bd}	11.27*	0.70	1.51	2.09
SP (mg/g)	6.41	3.10^{bd}	2.12^{bd}	2.28^{bd}	2.60^{bd}	4.66^{b}	5.82*	0.73	1.57	2.17
TT (mg/g)	6.17	4.90^{bd}	1.94^{bd}	3.78^{bd}	3.40^{bd}	2.30^{bd}	5.45^{b}	0.31	0.67	0.93

TABLE 2
Effect of biological treatments on quality attributes of maize stover

Nutritional parameters	Control	*T. harzianum*	*T. viride*	*S. cerevisae*	*P. fluorescens*	SE	CD (5%)	CD (1%)
CP (%)	8.57	12.25^{ac}	9.98^{ac}	10.50^{ac}	12.78^{ac}	0.42	0.93	1.33
TC (%)	13.04	8.69^{bd}	16.65^{ac}	8.58^{bd}	7.92^{bd}	0.40	0.89	1.27
RS (%)	6.24	6.31	6.85	5.90	4.29	1.22	NS	NS
NDF (%)	69.00	65.00	67.00	67.10	67.00	1.14	NS	NS
ADF (%)	46.20	45.97	44.03	46.00	43.70	2.16	NS	NS
IVDMD (%)	59.40	70.00^{ac}	69.40^{ac}	65.40^{ac}	66.60^{ac}	0.41	0.91	1.30
ADL (%)	4.70	3.60^{bd}	2.00^{bd}	4.20^{bd}	2.90^{bd}	0.15	0.35	0.48
HC (%)	22.80	19.03	22.97	21.10	23.30	2.28	NS	NS
Cel (%)	41.50	42.37	42.03	41.80	40.80	2.17	NS	NS
Ash (%)	3.80	2.50	3.15	2.50	3.15	0.72	NS	NS
Silica (%)	1.90	1.60^{bd}	1.20^{bd}	1.00^{bd}	1.30^{bd}	0.09	0.20	0.29
TP (mg/g)	12.58	8.25^{bd}	8.69^{bd}	9.96^{bd}	8.26^{bd}	0.39	0.87	1.24
SP (mg/g)	6.41	3.25^{bd}	3.64^{bd}	4.10^{bd}	2.36^{bd}	031	0.68	0.98
TT (mg/g)	6.17	5.00^{b}	5.05^{b}	5.86*	5.90*	0.40	0.89	NS

In table (s) 1 and 2, superscripts 'a' and 'b' represents significantly higher and significantly lower values as compared to untreated, respectively at 5% level of significance, whereas 'c' and 'd' represents significantly higher and significantly lower values as compared to untreated, respectively at 1% level of significance. NS: Non-significant, *: Significantly at improves the CP content (Katoch *et al.,* 2017). The increase in CP content of acid treated maize stover may be attributed to the acid precipitation of protein and its immobilization.

Carbohydrates are considered as readily available source of energy for animals. Among chemical treatments, the highest significant increase (57.90%) in total carbohydrates content of stover was observed with calcium hydroxide treatment, whereas acid hydrolysis and sodium hydroxide treatment resulted 3.70% and 5.90% increase, respectively. The increase in total carbohydrates content in alkali treated maize stover may be due to hydrolysis of glycosides and other complex carbohydrates (Hendriks and Zeeman, 2009). Acid hydrolysis increased the reducing sugar content of maize stover non-significantly (25.80%) while calcium hydroxide and sodium hydroxide treatments revealed 23.40% and 23.10% increase, respectively (Table 1). Chen *et al.* (2009) observed that when corn stover treated with dilute sulfuric acid (1.50%), it converted 76.60% of hemicellulose to soluble sugar. Lloyd and Wyman (2005) also reported that acid hydrolysis increases the xylose and glucose yield from subsequent enzymatic digestion of cellulose and hemicellulose.

With all biological treatments, the CP content increased significantly among which *Pseudomonas fluorescens* treatment resulted highest significant increase (Table-2). Islamiyati *et al.* (2013) reported 27.58% increase in CP content of maize stover inoculated with *Trichoderma sp. RI-7.* Treatment of maize stover with oyster mushroom *(Pleurotus ostreatus)* also resulted in significant increase in CP content (39.50% to 171.89%) (Adenipekun and Okunlade, 2012). Fungal secretion of tannase enzyme may also attribute to increased CP content in feeds (Raghuwanshi *et al.,* 2014). Treatment of stover with *T. viride* exhibited significant increase of 27.70% in total carbohydrate content where the increase of 9.80% in reducing sugar content was non-significant (Table-2). The increase in reducing sugars may be because of production of cell wall degrading enzymes causing hydrolysis of wall polysaccharides.

Fibre content : The fiber content (ADF and NDF) in forages has direct relationship with digestibility of feeds in livestock. High fiber content reduces the voluntary intake and amount of digestible energy from feedstuff. In the present study, alkali treatments were more effective in reducing the NDF and ADF contents of maize stover. The highest significant reduction in NDF content (8.80%) was observed with calcium hydroxide treatment, while sodium hydroxide treatment resulted highest (7.35%) decrease in ADF content (Table 1). Casperson *et al.* (2018) reported that treating corn stover with calcium hydroxide reduced the ADF and NDF contents of corn stover by 14% and 29%, respectively. The reduction in NDF content with alkali treatment in fibrous feeds may be due to increased solubilization of hemicellulosic fractions in cell wall.

Among biological treatments, inoculation with *T. harzianum* reduced the NDF content by 5.79% in maize stover. The reduction of 7.94 % in NDF content of maize stover has also been reported by Islamiyati *et al.* (2013) after inoculation of *Trichoderma sp*. Treatment with *P. fluorescens* revealed highest reduction (5.40%) in ADF content (Table-2). Yalchi and Hajighrari (2010) also observed significant reduction in NDF and ADF content in wheat straw inoculated with *T. harzianum* isolates. Fazaeli *et al.* (2004) reported 11.74% and 12.42% reduction in NDF and ADF content of rice straw inoculated with *Pleurotus* fungi, respectively. Treatment of maize stover with oyster mushroom (*Pleurotus ostreatus)* resulted 11.00% to 14.50% decrease in NDF content (Ramirez- Bribiesca *et al.,* 2010).

Cell wall constituents : Forage digestibility has negative relationship with the level of cell wall constituents *i.e.,* cellulose, hemicellulose and acid detergent lignin (ADL) (Katoch *et al.,* 2018). The ADL is one of the main constituent of plant cell wall that limits rumen microorganism utilization of cellulose and hemicellulose (Van Kuijk et al. 2015). In the present study, ADL content of stover reduced significantly (55.30%) with calcium hydroxide treatment, followed by urea and sodium hydroxide treatments (Table-1). Acid hydrolysis resulted in least reduction of 14.10% in ADL content (Table 1). Chen *et al.* (2009) also recorded 73.90% reduction in lignin content in maize stover treated with sodium hydroxide treatment (2%). Casperson *et al.* (2018) reported 20% delignification in corn stover treated with calcium hydroxide. Alkali treatment causes swelling, increasing the internal surface of cellulose and decreasing degree of polymerization and crystallinity which provokes delignification (Saritha *et al.,* 2012; Wang *et al.,* 2013). Among all chemical treatments, calcium hydroxide reduced the hemicellulose content by 14.00%, whereas, dilute sulfuric acid was not much effective with only 6.10% reduction (Table 1). Chen *et al.* (2009) also reported that lime treatment hydrolysed 14.80% of hemicellulose to soluble sugar from corn stover. The decrease in hemicellulose content of maize stover might be due to solubilization of hemicellulosic fractions in cell wall (Saritha *et al.,* 2012). Sodium hydroxide decreased the cellulose content by 3.40% (Table 1).

With all biological treatments, the ADL content in maize stover reduced significantly of which *T. viride* exhibited highest significant reduction (57.40%) (Table-2). Garcia-Torreiro *et al.* (2016) reported 45.80% delignification of corn stover with white-rot fungus *Irpex lacteus* for 21 days. Abdel-Azim *et al.* (2011) also observed decrease (11.19 to 10.24%) in ADL content of maize stalks inoculated with *T. viride*. The reduction in lignin content of 37.46% and 31.42% in wheat straw has also been observed with *Pleurotus ostreatus* and *Trametes versicolor* treatments, respectively (Shrivastava *et al.,* 2011). The reduction in lignin content may be attributed to lignin degrading enzymes in fungal secretions (Nerude and Misurocava, 1995). Hemicellulose and cellulose content of stover reduced non-significantly by 16.50% and 1.60% with *T. harzianum and P. fluorescens* treatments, respectively (Table-2). Xu et al. (2017) reported 47%, 39% and 55% reduction in lignin, hemicellulose and cellulose content in corn stover treated with *Inonotus obliquus*. Abdel-Azim *et al.* (2011) also reported decrease in hemicellulose and cellulose content of maize stalks treated with *T. viride*. The inoculation of different microorganisms to feed sources disrupts ligno-cellulosic complex and increases the accessibility of hydrolytic enzymes on cellulosic polysaccharides (Ramirez Bribiesca *et al.,* 2010).

***In vitro* dry matter digestibility (IVDMD) :** Stover digestibility revealed improvement after different pre-treatments. Treatment with sodium hydroxide revealed highest increase (16.83%) in digestibility followed by aqueous ammonia treatment (16.20%). The least improvement (4.70%) in digestibility was observed with salt treatment (Table 1). The solvation and saponification processes during alkali treatments alter the structure of plant fiber subsequently causing swelling of biomass and yield of fermentable sugars for rumen fermentation, thus increases the digestibility of stover (Casperson *et al.,* 2018).

The *in vitro* dry matter digestibility of stover increased with all biological treatments of which *T. harzianum* treatment revealed highest increase of 17.80% (Table-2). The increase in digestibility (25.89% to 30.72%) of wheat straw has also been observed with the inoculation of different isolates of *Trichoderma* species (Yalchi and Hajieghrari, 2010). Zuo *et al.* (2019) reported improvement in rumen degradability of corn stover after fermentation with *Irpex lacteus* fungu*s*. Khonkhaeng and Cherdthong (2020) also reported improvement in digestibility of purple field corn stover fermented with *P. ostreatus* and *Volvariella volvacea.* The increase in IVDMD might be due to decrease in fiber and cell wall constituents that mainly limit the digestibility of crop residues (Yalchi and Hajieghrari, 2010).

Polyphenolic compounds : Crop residues having high phenolic content are not considered consumer friendly as they result in impaired nutritional quality, lower digestibility and reduction of feed intake in animals. Tannins bind with nutrients irreversibly in GI tract and reduce their bioavailability to animals. With all chemical treatments, total phenols, simple phenols and total tannin content of maize stover reduced of which ammonium hydroxide treatment caused 67.70%, 66.90% and 68.60%, reduction, respectively (Table-1). Among biological treatments, the total phenolic content of stover reduced maximally (34.40%) when treated with *T. harzianum* followed by *P. fluorescens* (34.20%). The decrease in phenols may be due to its utilization as sole source of carbon and energy by *P. fluorescens* (Mahiudddin *et al.,* 2012) while, simple phenolic content exhibited 59.40% reduction, when inoculated with *P. fluorescens*. Treatment with *T. harzianum* was effective in reducing the total tannins content (18.90%) in maize stover (Table-2).

Ash and silica content : Ash content is defined as the inorganic residue left after incineration. The ash content of feed resources represents total mineral constituents of feed which play an important role in animal nutrition (Katoch *et al.,* 2018). In the present study, the calcium hydroxide treatment significantly increased the ash content (43.40%) in maize stover while sodium chloride, ammonium hydroxide and sodium hydroxide treatment were statistically similar. Casperson *et al.* (2018) reported 160% increase in ash content of corn stover treated with calcium hydroxide. An increase in ash content has also been reported by Nguyen *et al.* (2011) in rice straw treated with lime. Treatment with ammonium hydroxide (10%), the silica content in maize stover reduced significantly to the extent of 52.63% (Table-1). None of the biological treatment was effective in improving the ash content of maize stover. Treatment with *S. cerevisae* resulted highest significant decrease (47.40%) in silica content (Table-2). Fungal and bacterial treatments could solubilize the insoluble silicates (Umamahesweri *et al.,* 2016), resulting in the reduction of silica content of maize stover.

CONCLUSION

Crop residues are important dietary component during the periods of scarcity of green fodder and maize stover is one of them. The utilization of stover in livestock feeding is limited by low voluntary intake and low digestibility. The results unveiled the significant impact of chemical and biological treatments on quality and digestibility of stover that may be due to alterations in nitrogen content and breaking the complex bonds of fiber structure and other rigid complexes. The study concluded that treatment with alkali and *T. harzianum* and *P. fluorescens* were promising for improving quality and digestibility of stover. However, further studies are required for assessing the effect of feeding of treated stover on animal health and production.

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