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Improvement in the Quality Attributes of Forage Cowpea by Use of Liquid Microbial Inoculants

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ABSTRACT

Background: The present investigation was accepted to study the impact of liquid microbial inoculants on the quality parameters of forage cowpea (*Vigna unguiculata*). There upon, the field experiment was conducted during *kharif* 2018 at Punjab Agricultural University, Regional Research Station, Bathinda and at Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab respectively.

Methods: The experiment was laid out in randomized complete block design with a total of eleven treatment combinations of liquid microbial inoculants (*Burkholderia seminalis, Burkholderia* sp. and *Bradyrhizobium* sp.) with 75% and 100% RDF and replicated thrice. The qualitative properties including crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), *in-vitro* dry matter digestibility (IVDMD), total soluble sugars and ash content were determined on the forage samples.

Result: The results indicated that the treatment T_{10} (75% RDF + *Burkholderia* sp. + *Burkholderia seminalis*) has reduced ADF% and NDF% at both the locations. Forage with high ADF and NDF% content tends to have low digestibility, low ADF and NDF% is desirable. In addition, treatment T_{10} showed significantly ($p<0.05$) improved IVDMD (79.98 %) and total sugars (15.82 mg/g) at Ludhiana and at Bathinda, T_{10} treatment significantly escalated crude protein (19.93%), total ash content (11.55%), total chlorophyll content (2.07 mg/g) and total sugars (15.80 mg/g).Further correlation studies showed highly significant positive correlation among ADF% and NDF% and there negative correlation with all other quality components at both the locations. Consequently, liquid microbial inoculants can play pertinent role in ameliorating fibre quality and interrelation between the various quality parameters proposed that forage with low ADF% and NDF% can be selected for simultaneous amelioration in different attributes of quality.

Key words: *Bradyrhizobium*, *Burkholderia seminalis*, *Burkholderia* sp., Fodder cowpea, Liquid microbial inoculants, Qualitative properties.

INTRODUCTION

India is having the richest livestock population of 520 million heads, which is about 20 per cent of the world's livestock population. Livestock contributes nearly 4% to National GDP and it is source of employment to about 70% population in rural areas. Sustainable production of quality forages in ample quantities is imperative for profitable livestock productivity (Oguz and kaya, 2016). Thereupon, forage legumes have more important role owing to their superior quality than grasses and the ability to fix atmospheric nitrogen.

Among the leguminous fodder, cowpea [*Vigna unguiculata* (L.) Walp.] has emerged out as a potential crop for meeting the requirement of high quality fodder to fast expanding cattle population. In addition it fixes atmospheric nitrogen and contributes to soil fertility improvement. In Punjab, cowpea is predominantly grown for fodder starting from March to July. Due to its fast growing nature, it supplies nutritious and palatable fodder during *kharif* when there is scarcity of green fodder. In addition, cowpea forage is superior in higher protein contents and dry matter digestibility, thereby enhancing fattening of animals along with improving milk production.

Further, forage digestibility is related to chemical compositions particularly of fibre, lignin and crude protein to some extent. Generally, fibre concentration of the forage crops increases while quality and digestibility decreases as aging prolongs. Acid detergent fibre (ADF) and neutral detergent fibre (NDF) are commonly used as standard forage testing techniques for fibre analysis. ADF can be used to calculate digestibility, while intake potential is predicted through NDF (Ball *et al.,* 2001).

Nonetheless, the demand for huge quantity of chemical fertilizers and linked environmental tribulations have forced us to think on the urgent need of increasing good quality forage supply by adopting improved agronomic techniques.

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Thus, the situation accelerates the use of non-conventional approaches, such as an emerging trend in modern-day agriculture is the use of PGPR to enhance crop growth, yield and quality. PGPR are important bacteria that are beneficial and reside in the rhizosphere. They colonize the roots of plants which results in rapid plant growth. PGPR can ameliorate the growth of plants through plethora of mechanisms such as plant growth regulators production, nitrogen fixation and by increasing uptake of plant nutrients and water. In the neoteric era, myraid of PGPRs are being used in the form of inoculants to escalate the productivity, nutrients availability and quality of crops. Microbial inoculants are the formulations composed of beneficial microorganisms that play important role in the ecosystem. Keeping the aforesaid points in view the present research work has been carried out to study the influence of liquid microbial inoculants on the quality traits of forage cowpea.

MATERIALS AND METHODS

Procurement of test cultures

Three bacterial cultures were evaluated in this study. *Burkholderia seminalis* an endophytic bacteria was isolated from the root nodules of the vegetable cowpea [*Vigna unguiculata* (L.) Walp] plants and obtained from the Electron Microscopy and Nanoscience Laboratory, Department of Soil Science, PAU, Ludhiana, India. The other bacteria, *Burkholderia* sp., was isolated from the decomposed paddy straw and obtained from the Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India. The molecular identification of this culture showed similarity with *Burkholderia* sp. STM1441 based on nucleotide homology and phylogenetic analysis. The reference bacterial culture, *Bradyrhizobium* sp. (NAIMCC-B-00260) was obtained from the National Agriculturally Important Microbial Culture Collection. ICAR-National Bureau of Agriculturally Important Microorganisms (ICAR-NBAIM), Kushmaur, Mau Nath Bhanjan, Uttar Pradesh.

Experimental site

The field experiment was conducted at Punjab Agricultural University, Ludhiana (Forage and Research Farm) (30.9°N latitude and 75.85°E longitude and average elevation of 244 metres above sea level) and at Punjab Agricultural University, Regional Research Station, Bathinda (30°09″36′ N latitude, 74°55″28′ E longitude and at an altitude of 211 m above sea level).

Experimental details

The field experiment was laid out in RBD design and replicated thrice. There were eleven treatments, *viz*. T_1: Recommended dose of fertilizer (RDF), T_2: RDF + liquid microbial inoculant of *Burkholderia* sp., T_3: RDF + Liquid microbial inoculant of *Burkholderia seminalis* ,T_4: RDF + Liquid microbial inoculant of *Bradyrhizobium* sp.,T_5: RDF + Liquid microbial inoculant of *Burkholderia* sp. and *Burkholderia seminalis* respectively, T_6: RDF + Liquid microbial inoculant of *Burkholderia* sp. and *Bradyrhizobium* sp. respectively T_7: 75% of RDF + liquid microbial inoculant of *Burkholderia* sp.,T_8: 75% of RDF + Liquid microbial inoculant of *Burkholderia seminalis,* T_9: 75% of RDF + Liquid microbial inoculant of *Bradyrhizobium* sp., T_{10}: 75% of RDF + Liquid microbial inoculant of *Burkholderia* sp. and *Burkholderia seminalis* respectively and T_{11}: 75% of RDF + Liquid microbial inoculant of *Burkholderia* sp. and *Bradyrhizobium* sp. The land preparations were done mechanically with proper care to avoid mixing of soil from adjacent plots. Cowpea variety 'CL-367' was sown at the rate 25 kg/acre as per treatment schedule at an inter-row spacing of 30 cm with the gross plot dimensions of 5 m × 3m. Nitrogen and phosphorus were applied as per the treatments through urea and single super phosphate. Seed bacterization was done with liquid microbial inoculants of *Burkholderia seminalis, Burkholderia* sp. and *Bradyrhizobium* sp. as per treatments @ 100 ml/ acre. Inoculated seeds were then air dried in shade and planted within 2 hours. Weeding and hoeing was done to avoid weeds and suitable control measures were taken to prevent insects and pests. Other cultural operations and plant protection measures were followed as per the recommendations.

Forage quality attributes

The quality traits studied in the present study were acid detergent fibre(ADF), neutral detergent fibre (NDF) by the method of Goering and Vansoest (1970), crude protein (CP) content (Mckenzie and Wallace 1954), *in vitro* dry matter digestibility (IVDMD) (Tilley and Terry, 1963), ash content (AOAC, 1990), total sugars (Dubois *et al*., 1956).

Statistical analysis

A randomized complete block design with three replications was employed for data analyses. All statistical analyses were performed by the procedure as described by Cochran and Cox (1967). The comparisons were made at 5% level of significance. The correlation between quality parameters of forage cowpea as influenced by various liquid microbial inoculant treatments was also studied. The correlation matrix was prepared by using corrplot library in R studio.

RESULTS AND DISCUSSION

Effects of liquid microbial inoculants on quality attributes of forage cowpea

It is imperative to improve the quality of fodder crops. Therefore, in the present study, effect of different treatments of liquid microbial inoculation on the nutritional quality of forage cowpea has been studied.

Acid detergent fibre (ADF)

Acid detergent fibre are frequently used as standard for evaluating the quality of forage. ADF primarily represents cellulose and lignin. Because of the negative relationship between the digestibility and ADF, low ADF is desirable.

The perusal of the data revealed significant reduction in ADF% in treatments with liquid microbial inoculants when

compared with the control (P≤0.005). Highest and the lowest ADF% was noted in control (T_1: RDF) and treatment T_{10} *i.e* 75% of RDF + *Burkholderia* sp.+ *Burkholderia seminalis* with values of 37.94% and 34.25% at Ludhiana respectively. The percentage decrease in ADF% over T_1 (control) in T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 was 9.25%, 8.90%, 8.59%, 8.40%, 6.41%, 3.60%, 0.74%, 0.61%, 2.28% and 0.56% respectively at Ludhiana.

Likewise, data pertaining to ADF (%) at Bathinda revealed decrease in ADF% with the application of liquid microbial inoculants (Table 2). The percentage decrease in ADF content over T_1 (control) in T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 was 12.67%, 12.21%, 11.78%, 10.63%, 10.27%, 6.39%, 2.14%, 2.97%, 1.67% and 1.29% respectively at Bathinda location.

In all the treatments with liquid microbial inoculants decline in ADF % was recorded and lowest ADF% was recorded with treatment 75% of RDF + *Burkholderia* sp. + *Burkholderia seminalis.* This might be due to escalated availability of nitrogen owing to biological nitrogen fixation by *Burkholderia* sp, *Burkholderia seminalis* and *Bradyrhizobium* sp. Improved uptake of nitrogen imparts succulence to green plants by reducing the fibre content.

These results are in close conformity with the earlier findings of Asefa (2018) who reported that the application of *Rhizobium* inoculants and phosphorus fertilizer had significant negative effects on haulm NDF, ADF and ADL content of faba bean over control.

Neutral detergent fibre (NDF)

NDF consists of slowly digestible and non-digestible fibrous portion of plant which commonly consists of cell wall material including hemicellulose. However, if NDF of the ration is too low it results in health problems like displaced abomasum, acidosis and foundering may occur (Ball *et al.,* 2001).

Data presented in Table 1 showed non-significant effect of liquid microbial inoculants on NDF% (P≤0.005). However, numerically highest and lowest NDF% was recorded in control (T_1: RDF) and treatment T_{10} *i.e* 75% of RDF + *Burkholderia* sp. + *Burkholderia seminalis* (54.41% and 48.18% respectively) at Ludhiana. The percentage decrease in NDF% over T_1 (control) in T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 was 11.45%, 11.13%, 11.13%, 9.13%, 7.51%, 7.48%, 5.64%, 5.58%, 5.53% and 2.07% at Ludhiana respectively.

The data pertaining to NDF% at Bathinda showed significant effect with liquid microbial inoculants (Table 2).

Table 1: Effect of liquid microbial inoculants on forage cowpea quality attributes at Ludhiana location.

Treatments	ADF (%)	NDF (%)	CP (%)	IVDMD (%)	Total ash (%)	Total sugars (mg/g)
T1	37.94	54.41	17.28	71.15	10.22	14.97
T2	36.88	51.4	18.39	72.98	10.43	15.21
T3	37.51	51.37	17.75	73.14	12.01	15.25
T4	37.53	53.28	18.33	72.76	10.42	15.16
T5	34.38	48.35	20.07	79.62	12.59	15.73
T6	34.57	49.44	18.99	76.59	12.53	15.62
T7	36.38	50.34	18.38	74.9	12.14	15.57
T8	35.32	50.32	18.45	76.04	12.19	15.77
T9	37.46	51.34	17.88	74.58	12.06	15.33
T10	34.25	48.18	20.11	79.98	12.78	15.82
T11	34.5	48.35	19.75	80.04	12.54	15.64
CD (5%)	1.77	NS	NS	3.15	NS	0.29

Table 2: Effect of liquid microbial inoculants on forage cowpea quality attributes at bathinda location.

Treatments	ADF (%)	NDF (%)	CP (%)	IVDMD (%)	Total ash (%)	Total sugars (mg/g)
T1	38.37	54.79	16.71	66.32	9.11	14.42
T2	37.73	52.22	16.85	70.18	9.37	14.63
T3	37.23	51.88	17.91	70.35	9.3	15.16
T4	37.88	53.71	16.77	67.35	9.14	14.62
T5	33.68	47.18	19.72	72.76	11.29	15.78
T6	34.29	48.42	19.19	72.44	10.55	15.57
T7	35.92	50.61	18.8	70.42	10.23	15.01
T8	34.43	49.16	18.97	71.22	10.26	15.72
T9	37.55	50.68	18.06	69.67	9.55	15.3
T10	33.51	47	19.93	74.46	11.55	15.8
T11	33.85	47.26	19.55	72.52	10.48	15.62
CD (5%)	3.3	2.82	1.4	NS	1.26	0.25

Maximum and minimum NDF% of 54.79% and 47.00% was observed in control (T_1: RDF) and T_{10} treatment (75% of RDF + *Burkholderia* sp. + *Burkholderia seminalis*) respectively. However, treatments T_2 and T_4 were at par with treatment T_1whereas treatments T_3, T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11} showed significant reduction in NDF% over T_1. The percentage decrease in NDF% over T_1 (control) in T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 was 14.22%, 13.89%, 13.75%, 11.63%, 10.28%, 7.64%, 7.51%, 5.31%, 4.69% and 1.97% at Bathinda respectively.

The reduction in the NDF might be due to increased succulence by reducing the formation of polysaccharides such as lignin, cellulose and hemicellulose which generally account for NDF content in plant because of biological nitrogen fixation by *Burkholderia seminalis*, *Burkholderia* sp.and *Bradyrhizobium* sp. These results are in agreement with the findings of Patel *et al.* (2012) who reported that increased supply of N fertilization and other minerals resulted in decreased levels of NDF content in fodder maize.

Crude protein (CP)

Dietary proteins are vital constituents of animal feed and its nutritional and functional properties affect the overall quality of the feed. As a legume, cowpea is a rich and low-cost source of proteins and nutrients (Egounlety and Aworh, 2003). Crude protein is an estimate for the total proteinThe data showed non-significant effect of liquid microbial inoculant on crude protein content in all treatments at Ludhiana location (Table 1). Nevertheless, the percentage increase in crude protein with respect to T_1 in treatment T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 was 16.38%, 16.14%, 14.29%, 9.90%, 6.77%, 6.42%, 6.37%, 6.08, 3.47% and 2.72% respectively.

However, at Bathinda, crude protein content significantly increased over the control in all treatments of liquid microbial inoculants (Table 2). Highest crude protein content was recorded with T_{10} treatment .The percentage increase in crude protein content of treatments T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 over control are 19.27%, 18.01%, 17.00%, 14.84%, 13.52%, 12.51%, 8.08%,7.18%, 0.84% and 0.36% respectively. Treatments T_{11}, T_{10}, T_8, T_7, T_6 and T_5 showed significant improvement in crude protein content and treatments T_2, T_3, T_4 and T_9 were at par with treatment T_1.

This might be due to improved growth due to phytohormones production by the inoculated bacteria which stimulate nutrients absorption as well as photosynthesis process, as a result of which protein content increases. This is in agreement with the finding of Tesfaye *et al*. (2018) who reported significant improvement of soybean crude protein content due to application of *Bradyrhizobium* inoculants.

***In vitro* dry matter digestibility (IVDMD)**

IVDMD is a very important and most demanded key quality traits for fodder production where it governs nutrient availability to animal for their health and growth. Liquid microbial inoculants showed significant effect on IVDMD content in all treatments at Ludhiana location (Table 1). The percentage increase in IVDMD over T_1 in treatment T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 was 12.49%, 12.41%, 11.90%,7.65 %, 6.87%, 5.27%, 4.82%, 2.80%, 2.57% and 2.26% respectively. Moreover, treatments T_2, T_3 and T_4 were at par with treatment T_1 and treatments T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11} showed significant improvement in IVDMD content over T_1. However, IVDMD content was non-signficantly affected by all the treatments of liquid inoculants at Bathinda. Accordingly, the percentage increase in IVDMD content of treatments T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_2, T_3 and T_4 over control (T_1) was 12.27%, 9.71%, 9.35%, 9.23%, 7.39%, 6.18%, 6.08%, 5.82%, 5.05% and 1.55% respectively. Positive response of IVDMD to the application of different inoculant treatments (*Burkholderia seminalis*, *Burkholderia* sp and *Bradyrhizobium* sp.) might be due to the phosphate solubilizing activity of the inoculated cultures.Further, improved availability of phosphorus could be considered as an important means to improve the nutritional value of cowpea forage. These findings were in line with earlier studies of Reddy *et al.* (2003) who reported that inoculation of cereal seed with nitrogen fixing bacteria such as *Azotobacter* and seed of pulses with phosphate solubilizing bacteria resulted in decreased fertilizer requirement and improved crude protein and IVDMD (*In vitro* dry matter digestability) of fodder.

Ash content

The ash content of feed resources (%) non-significantly influenced by the different treatments of liquid microbial inoculants**.** In our study highest ash content was observed with T_{10} followed by T_5. The percentage increase in ash content over T_1 was 25.05%, 23.19%, 22.70%, 22.60%, 19.28%, 18.79%, 18.00%, 17.51%, 2.05% and 1.96% in T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_3, T_2 and T_4 respectively at Ludhiana (Table 1). At Bathinda location, liquid inoculants had significant effect on ash percentage (Table 2) and ash content varied from 9.11% (T_1) to 11.55 % (T_{10}). Moreover, the treatments T_2, T_3, T_4, T_7, T_8 and T_9 were at par with T_1 whereas treatments T_5, T_6, T_{10} and T_{11}showed significant improvement in total ash content. The percentage increase in ash content over T_1 was 26.78%, 23.93%, 15.81%, 15.04%, 12.62%, 12.29%, 4.83%, 2.85%, 2.09% and 0.33% in T_{10}, T_5, T_{11}, T_6, T_8, T_7, T_9, T_2, T_3 and T_4 respectively. The increase in total ash percentage in treatments with liquid microbial inoculants might be due to the production of growth promoting hormones such as auxin, cytokinin and gibberellin through *Burkholderia seminalis*, *Burkholderia* sp. and *Bradyrhizobium* sp. and improved growth due to mineral nutrient uptake from the soil which in turn might have resulted in increased total ash content. This was in line with those Shahverdi *et al.* (2014) who reported that native *rhizobium* with reduced application of chemical fertilizers treatment as compared to no application of biological fertilizers with recommended chemical fertilizers showed increased ash percentage (3.25%) in Persian clover (*Trifolium rosapinatum* L.).

Total sugars

Data regarding the total sugar content has been presented in Table 1 and Table 2. The application of liquid microbial inoculants significantly increased total sugar content in leaves over the control at Ludhiana (Table 1). However, maximum total sugar content was recorded in T_{10} (15.82 mg/g) while minimum total sugar content was noted in the T_1 treated plants (14.97 mg/g). However, the treatments T_3,T_5, T_6, T_7, T_8, T_4,T_9, T_{10} and T_{11} improved significantly over the treatment T_1.Similarly, significant increase in the total sugars content was observed in the treatments with liquid microbial inoculants at Bathinda location. Maximum total sugars were recorded by T_{10} treatment (15.80 mg/g) and minimum total sugar was recorded by T_1 treatment (14.97 mg/g). The treatments T_3, T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11} improved significantly and the treatments T_2 and T_4 were found to be at par with T_1.

The improved sugar content due to supplementation with microbial inoculants might be due to better absorption and mobilization of nutrients by the action of *Burkholderia seminalis, Burkholderia* sp. and *Bradyrhizobium* sp.The results were in conformity with Stancheva *et al.* (2016) who reported that *Bradyrhizobium japonicum* solely and in combination with *arbuscular mycorrhizal* fungi increased the content of proteins, soluble sugars and total phenols in the cowpea.

Correlation analysis of quality attributes

Correlation analyses are being widely used in many crop species by the plant breeders to understand the nature of complex interrelationships among the traits and to identify the sources of variation in yield (Finne *et al.,* 2000).

The data pertaining to the correlation between the quality components as influenced by the liquid microbial inoculants has been presented in Fig 1 for Ludhiana location and Fig 2 for Bathinda location. Interpretation of data revealed positive correlation between ADF% and NDF%. However, both these parameters were found to be negatively correlated with other quality parameters at both the locations. This might be due to the fact that lignin and cellulose component of ADF are also component of NDF. Eskandari *et al.* (2009) also reported that acid detergent fibre and neutral detergent fibre are major indicators of digestibility which negatively affects feed quality. Further, in present investigation liquid microbial inoculants were found to reduce the ADF and NDF percentage.

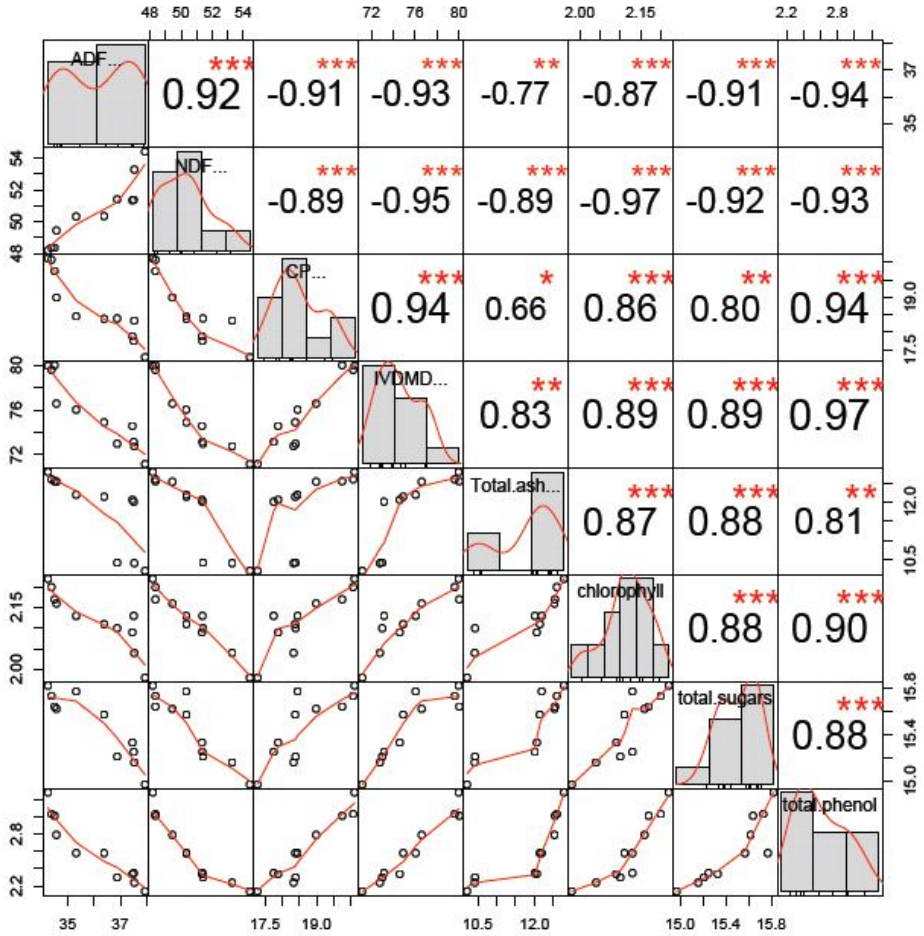

Fig 1: The correlation between quality attributes of forage cowpea as influenced by various liquid microbial inoculants treatments at Ludhiana.

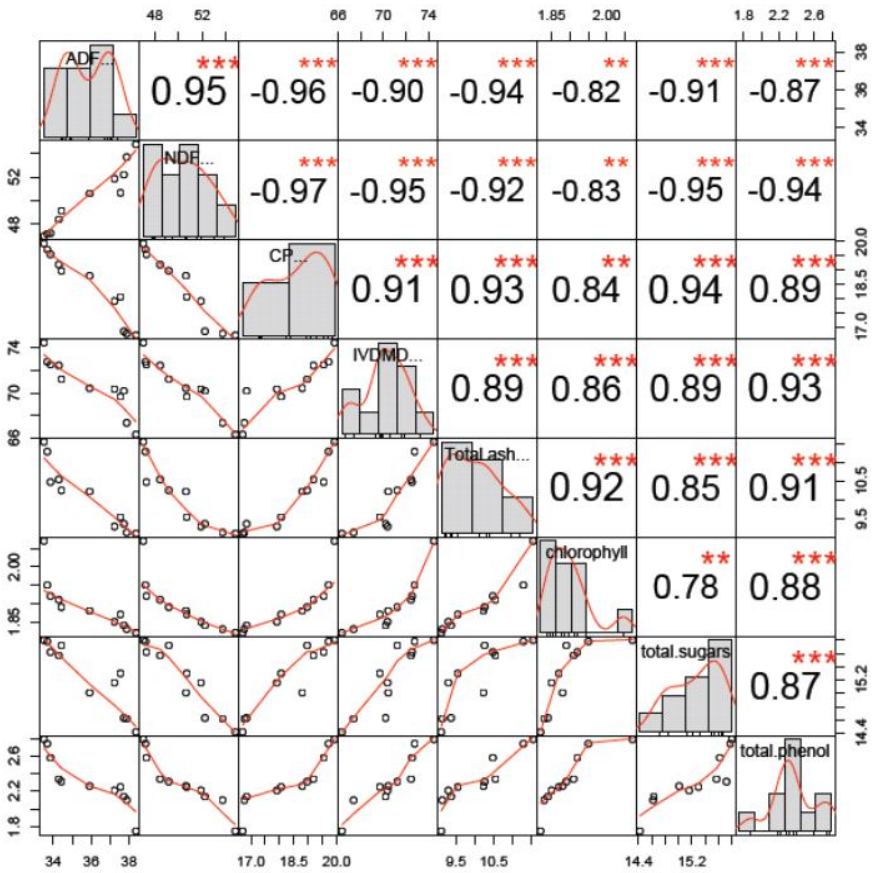

Fig 2: The correlation between quality attributes of forage cowpea as influenced by various liquid microbial inoculants treatments at Bathinda.

Likewise, crude protein component of forage cowpea found to exhibit significant positive correlation with IVDMD, chlorophyll content, and total phenols whereas at Bathinda location, CP found to be significantly correlated with IVDMD, total sugars and total phenols. Similarly, IVDMD found to be significantly correlated with chlorophyll, total sugars and total phenols at Ludhiana location and at Bathinda location, it was found to be significantly correlated with total ash in addition to chlorophyll, total sugars and total phenols. Similar results were seen by Anup and Vijaykumar (2000). IVDMD were also found to have positive significant correlation with total sugars and total ash content. These results were at par with Kaur and Thakur (2016) who reported that IVDMD showed positive association with carbohydrate fractions (TCHO) of energy feeds.

Total ash content found to be significantly correlated with chlorophyll, total sugars and phenolic content at both the locations. Furthermore, chlorophyll content of the leaves was found to significantly correlate total sugars and phenol at Ludhiana location and at Bathinda location, it was only significantly correlated with total phenols. Further at both the locations, total sugars and total phenols found to be significantly correlating with each other.

The results of correlation studies revealed that ADF and NDF had negative correlation with CP indicating that the higher CP would suggest lower fibre content in fodder crops and could be taken as one of the selection criteria. Our results were in accordance with previous studies on ADF content in cowpea (Devasena *et al.,* 2009) and NDF content in cowpea (Prusty *et al.,* 2013). Thus, the use of liquid microbial inoculants resulted in increased CP and IVDMD content and lowered ADF and NDF content. Thereupon, ascertaining their positive role in improving cowpea fodder quality traits. Turk *et al.* (2015) also reported that the selection for increased crude protein would result in lower fibre concentration which would be desirable from the standpoint of forage quality. Maximum improvement observed in the quality paramenters was with 75% of RDF + *Burkholderia* sp. + *Burkholderia seminalis* followed by 100% RDF+ *Burkholderia* sp. + *Burkholderia seminalis*.

CONCLUSION

Microbial inoculants could play predominant role in improving forage quality. In addition, highly significant correlation was observed between various quality parameters. It is reckoned that selection for one quality parameter can be useful in simultaneous improvement in the other quality attributes.

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Conflict of Interest

Authors S. Ramya, Gulab Pandove, Harpreet Oberoi, Sukhdeep Kaur and Anu Kalia declare no conflict of interest.

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