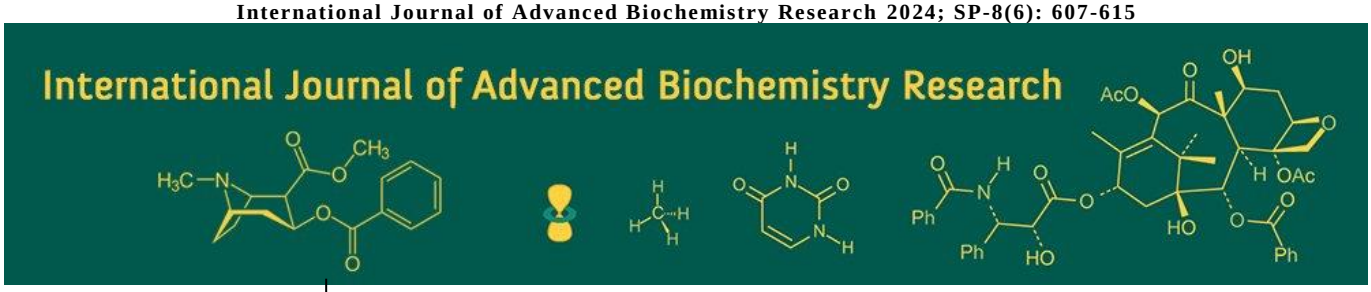

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Effect of enzymes and bacterial inoculant and their combination on the fermentation parameter and aerobic stability of maize silage

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Abstract
The experiment aimed to assess and compare the effect of bacterial inoculants, enzymes and their combinations on the fermentation process and aerobic exposure deterioration rate of maize silage. Maize fodder was harvested at one-third to half milk line stage (average dry matter 30.62%). Additive were used on a fresh matter basis for silage preparation. The treatments were control (no additive), cellulase, (6000 NCU/kg), xylanase (1500 IU/Kg[1]), C+X, *Lactiplantibacillus plantarum* (LP @ $1*10^6$ CFU g^{-1}), LP+C, LP+X, LP+C+X, *Limosilactobacillus fermentum*, (LF @ $2*10^6$ CFU g^{-1}), LF+C, LF+X, LF+C+X. Silage fermentation parameters and aerobic stability were evaluated after 30 days of ensiling. The addition of additives resulted in increased levels of lactic acid, acetic acid and dry matter recovery, accompanied by a decrease in pH. Notably, the LP+C+X treatment exhibited significantly higher lactic acid concentration (7.63) compared to other treatments. During aerobic exposure on different days, silage treated with combinations like LF and their respective combination demonstrated the lowest pH, yeast and mould counts. In summary, these results indicate that the additives and their combinations effectively improved quality indicators. However, the combination including the heterofermentative bacterial inoculant proved most effective in mitigating silage deterioration during aerobic conditions.

Keywords: Bacterial inoculant, enzymatic additive, fermentation parameter, aerobic stability, maize silage

Introduction
Silage is becoming increasingly popular in various regions of India due to its ability to provide a consistent supply of roughage throughout the year. The basis of ensiling phenomena is the naturally occurring anaerobic fermentation of fodder in the presence of lactic acid-producing bacteria, which convert readily fermentable carbohydrates into organic acids, primarily lactic acid (KocCoskuntuna and Ozduven, 2008) [26].

Ensiling is naturally a spontaneous process influenced by epiphytic microflora. However, the addition of silage additives can regulate and enhance fermentation, thereby improving silage quality and aerobic stability (Kung *et al*., 2003; Chauhan *et al*., 2021) [28, 10]. Various additive combinations are used to enhance fermentation parameters and aerobic stability.

Through analysis of multiple silage experiments, it has been observed that homofermentative lactic acid bacteria enhance dry matter recovery (DMR) during fodder fermentation, thereby improving the efficiency of the ensiling process.

It has been observed that silage high in carbohydrates or well-preserved silages with high lactic acid concentrations and low quantities of volatile fatty acids (acetic acid) are particularly susceptible to aerobic degradation (McDonald *et al*., 1991) [30]. Aerobic deterioration of silage primarily occurs due to the proliferation of yeasts and moulds, which lie dormant under anaerobic conditions and rapidly multiply upon exposure to oxygen. Obligatory heterofermentative lactic acid bacteria enhance silage aerobic stability through the production of acetic acid and by suppressing the growth of undesirable fermentation bacteria (Kleinschmit and Kung, 2006) [25]. Homofermentative bacterial inoculants improve the fermentation profile by increasing the production of lactic acid.

Enzymes can partially break down plant cell walls (cellulose and hemicellulose), releasing soluble sugars that lactic acid bacteria (LAB) can ferment, thereby lowering the pH of the silage. This partial digestion of the plant cell wall may enhance the speed and/or efficiency of digestibility (Dean, 2005) [16].

In the present study, an attempt has been made to improve the fermentation parameters and aerobic stability of silage through the addition of bacterial inoculant and enzymatic additive.

Materials and Methods

Silage preparation and additive treatment

The research was conducted at the National Dairy Research Institute in Karnal, Haryana, situated at an altitude of 250 meters above sea level, with coordinates of 29° 42" N latitude and 79° 54" E longitude. Maize fodder was harvested from the NDRI fields at the one-third to half milk line stage, with a dry matter content of 30.62%. The fodder was chopped using an electric chaff cutter to an average length of 1.5-2 cm and ensiled in a plastic container equipped with a lid that allows gas release and creates a vacuum. For silage preparation following additives were used on a fresh basis. The treatments were control (no additive), cellulase, (6000 NCU/kg), xylanase (1500 IU/Kg[1]), C+X, *Lactiplantibacillus plantarum* (LP @ $1*10^6$ CFU g^{-1}), LP+C, LP+X, LP+X+C, *Limosilactobacillus fermentum*, (LF @ $2*10^6$ CFU g^{-1}),), LF+C, LF+X, LF+X+C. Silage fermentation parameters and aerobic stability were evaluated after 30 days of ensiling.

Each treatment involved filling three containers with maize fodder, thoroughly mixed with additives to assess their individual or combined effects. These containers were tightly sealed, weighed and stored at room temperature. After 30 days of ensiling, samples were analyzed in triplicate to evaluate fermentation and quality parameters, microbial composition and aerobic stability. Pre-ensiled samples were collected for chemical analysis prior to the ensiling process.

Chemical composition, fermentation parameters and silage quality assessment

The chemical composition (DM, CP, OM and EE) of fresh maize fodder was determined as per the method described by AOAC (2005) [3]. The pH of fresh fodder and silage was determined using the Eutech pH meter from the aqueous extract. The water-soluble carbohydrate (WSC) content of fresh fodder was determined by a spectrophotometer after a reaction with an anthrone reagent. The fermentation coefficient (FC) of maize fodder was calculated using dry matter, water-soluble carbohydrates and buffering coefficient. The weight of the forage mass in the plastic container and its DM content at day 0 and 30th day were used to calculate the DM recovery (DMR) (da Silva *et al*., 2020). Lactic acid estimation was done as per the method described by Barnett (1951) [6]. Acetic acid was estimated with the help of Nucon's gas-liquid chromatography. Flieg point was calculated from the pH value and DM of silage at the end of the fermentation period with the following equation.

Flieg point = 220 +[(2*DM-15)]-40*pH

LAB counts were determined by pour-plating 10-fold serial dilutions of fresh maize fodder and silage samples on De Man, Rogosa, and Sharpe agar (De Man *et al*., 1960) [15] obtained from Himedia Laboratories Pvt Ltd, Mumbai, India. The Petri plates were then incubated at 37°C for 36 hours to enumerate LAB. Total counts of yeasts and molds were assessed by pour-plating 10-fold serial dilutions on potato dextrose agar, which was acidified with 0.5% (vol/vol) of 85% lactic acid after autoclaving. These plates were incubated aerobically for 48 hours at 25°C. The concentration of Clostridia spores in fresh silage samples was determined using the most probable number (MPN) method.

Fitness value (Davies *et al*., 2000) [14] were modified as follows for the present experiment conditions

Modified fitness value =

$$\left(\frac{1}{1+\left[\text{pH wtg}*\left(\frac{\text{pH}}{\text{cntlpH}}\right)\right]+\left[\text{DML wtg}*\left(\frac{\text{DML}}{\text{cntlDML}}\right)\right]+\left[\text{Amm}-\text{Nwtg}*\left(\frac{\text{AmmN}}{\text{cntlAmm}-\text{N}}\right)\right]}\right)$$

Weightage for different Parameters pH 4, DM Loss 3, Ammonia-N 3

Where wtg is weightage, cntl is control silage, DML is dry matter loss, and Amm-N is ammonia nitrogen content.

Aerobic stability

Aerobic stability was assessed by monitoring changes in pH and yeast and mould counts of silage during aerobic exposure. Approximately 2 kg of silage samples were placed in plastic buckets and stored at room temperature (25 °C). Samples were taken at intervals during aerobic exposure (0, 2, 4, 6 and 8 days) to measure pH and enumerate yeast and mould populations (Dolci *et al*., 2011) [17]. For yeast and mould enumeration, 11 grams of silage sample from each replicate were homogenized with 99 mL of sterile water. The homogenates were then plated on potato dextrose agar acidified with lactic acid. Yeasts and moulds were differentiated based on morphological characteristics. Plates with colony counts between 30 and 300 were used to determine colony-forming units (CFU).

Statistical design

The fermentation parameter data were analyzed using one-way analysis of variance (ANOVA) in SPSS (version 26.0) software. Aerobic stability data were subjected to a two-way analysis of variance, considering fixed effects of additives, ensilage period, and their interaction (additives × ensilage period). For LAB, yeast, and mould counts, logarithm base 10 transformations were applied to the data. Pairwise comparisons of mean values were conducted using Duncan's multiple range tests (Duncan, 1955) [18] with a significance level set at $p<0.05$.

Results

Chemical composition of maize green fodder

The composition of the maize fodder before ensiling (Table 1) shows the following values, dry matter (DM) 30.62%, crude protein (CP) 9.46%, ether extract (EE) 2.76% and total ash (TA) 6.05%. The pH of the maize fodder was 6.05 and its water-soluble carbohydrate content was 12.73%. The epiphytic LAB count in the maize fodder was 5.99 log10 CFU/g, while the yeast and mould count 3.20 log10 CFU/g.

Table 1: Chemical composition, fibre fraction, microbial count and energy content of fresh maize fodder

Items (%DM)	Maize fodder before ensiling	†SEM
Dry matter	30.62	0.40
Crude protein	9.46	0.02
Ether extract	2.76	0.08
Organic matter	93.95	0.50
Total Ash	6.05	0.04
pH	6.05	0.01
Water-soluble carbohydrate	12.70	0.03
Fermentation coefficient	40.58	0.50
Lactic acid bacteria (log10CFU/g)	5.99	0.05
Yeast and mould (log10CFU/g)	3.20	0.04

†SEM: Standard Error of Mean, CFU: Colony Forming Unit.

Table 2: Fermentation parameters of maize silage treated with the different additive combinations

Treatments	pH	Lactic acid (%DM)	Acetic acid (%DM)	L:A
Control	$4.16^{a}\pm0.02$	$6.23^{e}\pm0.27$	$1.81^{g}\pm0.48$	$3.44^{abc}\pm0.21$
C	$4.05^{b}\pm0.02$	$7.06^{bcd}\pm0.12$	$1.94^{ef}\pm0.04$	$3.65^{a}\pm0.12$
X	$4.06^{b}\pm0.01$	$7.00^{bcd}\pm0.25$	$1.891^{fg}\pm0.05$	$3.71^{a}\pm0.10$
C+X	$4.08^{b}\pm0.01$	$7.12^{bcd}\pm0.03$	$1.97^{def}\pm0.01$	$3.62^{a}\pm0.04$
LP	$4.04^{b}\pm0.02$	$7.14^{bcd}\pm0.03$	$2.03^{cde}\pm0.03$	$3.52^{ab}\pm0.05$
LP+C	4.04b±0.01	$7.44^{ab}\pm0.16$	$2.06^{bcd}\pm0.05$	$3.62^{a}\pm0.16$
LP+X	$4.07^{b}\pm0.05$	$7.37^{abc}\pm0.13$	$2.05^{bcde}\pm0.04$	$3.60^{a}\pm0.01$
LP+C+X	$4.02^{b}\pm0.01$	$7.63^{a}\pm0.19$	$2.09^{abc}\pm0.03$	$3.66^{a}\pm0.05$
LF	$4.09^{b}\pm0.01$	$6.66^{d}\pm0.06$	$2.11^{abc}\pm0.02$	$3.16^{c}\pm0.05$
LF+C	$4.02^{b}\pm0.04$	$7.08^{bcd}\pm0.08$	$2.18^{a}\pm0.05$	$3.25^{bc}\pm0.01$
LF+X	$4.07^{b}\pm0.01$	$6.93^{cd}\pm0.06$	$2.16^{ab}\pm0.02$	$3.21^{bc}\pm0.08$
LF+C+X	$4.03^{b}\pm0.01$	$7.23^{abc}\pm0.06$	$2.19^{a}\pm0.01$	$3.29^{bc}\pm0.02$
SEM	0.01	0.068	0.021	0.040
p-value	0.00	0.001	0.001	0.002

Values with different superscripts within a column differ significantly ($p<0.05$)

L: A is the lactic acid to acetic acid ratio; C-cellulase; X xylanase; C+X is the combinations of cellulase and xylanase; LP is the *Lactiplantibacillus plantarum*; LF is the *Limosilactobacillus fermentum*

Fermentation parameters of maize silage treated with varying additive combinations

The fermentation parameters of maize silage treated with various additive combinations were assessed after 30 days of ensiling (Table 2). The pH value showed a significant decrease ($p<0.05$) and lactic acid concentration increased in all treatment groups compared to the control. The control group exhibited the lowest lactic acid content (6.23%), whereas the highest (7.63%) was observed in maize silage treated with LP+C+X inoculation. Overall, the additives improved fermentation characteristics, notably pH and lactic acid levels. The acetic acid content was notably higher in silages treated with LF and their respective combinations than those treated with homofermentative bacterial inoculant and the control. Silages inoculated with heterofermentative bacteria and their combinations (LF, LF+C, LF+X, LF+C+X) exhibited elevated concentrations of acetic acid and a lower ratio of lactic acid to acetic acid compared to the homofermentative group and the control.

Effect of additives on microbial count

Figure 1 illustrates the variations in lactobacilli and yeast-mould counts in maize silage. The count of lactic acid bacteria was significantly higher ($p<0.05$) in additive-treated maize silage compared to the control. Yeast and mould counts ranged from 2.86 to 4.97 log10CFU/g. The lowest count was found in LF+C+X treated silage (2.86 log10CFU/g).

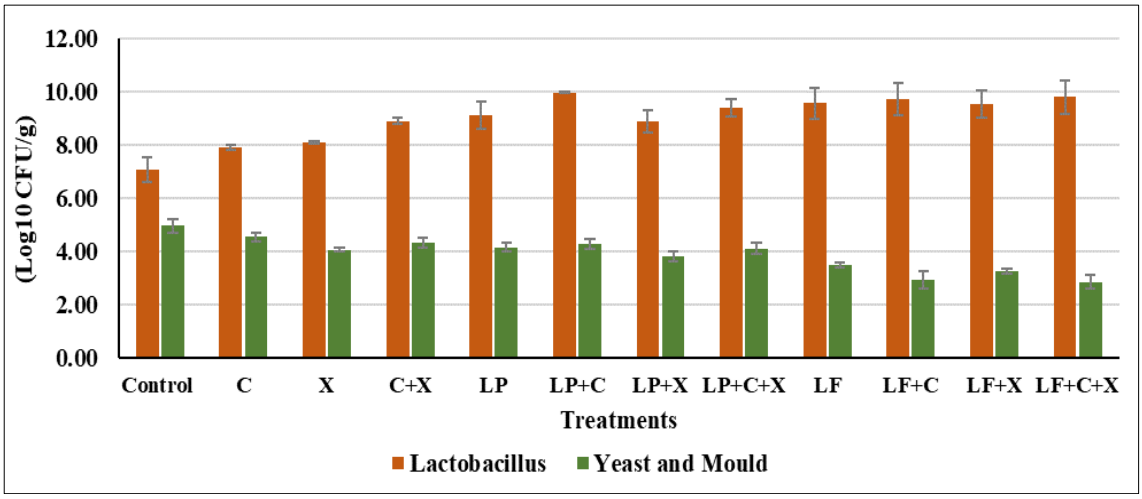


Fig 1: Microbial count of maize silage treated with various additive combinations

Quality parameters of maize silage treated with different additive combinations

Table 3 presents the modified fitness value, flieg point and dry matter recovery (DMR) of maize silage. Compared to the control, all additive-treated silages showed significantly higher values ($p<0.05$) for modified fitness, flieg point and DMR. The highest modified fitness value was observed in LP+X+C (0.1072), while the lowest was in the control (0.0909). The modified fitness value is influenced by pH, dry matter loss and ammonia nitrogen content of the silage. The modified fitness value is indicative of the efficacy of silage additives. Flieg points of all silages were significantly higher ($p<0.05$) than the control, although there were no significant differences among the treatments. The flieg point is determined by the pH and dry matter content of the silage. The dry matter recovery of maize silage ranged from 85.85% (control) to 88.79% (LP+C+X). This indicates that the additives used in this study effectively increased dry matter recovery. Treatment with inoculants containing LF led to lower dry matter recovery (DMR). This could be attributed to more extensive hetero-lactic fermentation and increased CO_2 production.

Table 3: Quality parameters of maize silage treated with varying additive combinations

Treatments	Modified Fitness value	Flieg point	DM recovery (%)
Control	0.0909^{d}±0.001	89.63^{b}±1.58	85.85^{b}±0.29
C	0.1028^{abc}±0.001	94.32^{ab}±2.09	87.11^{a}±0.29
X	0.1013^{bc}±0.001	94.67^{a}±0.60	87.25^{a}±0.31
C+X	0.1046^{ab}±0.001	93.71^{ab}±1.14	87.26^{a}±0.39
LP	0.1039^{ab}±0.001	96.47^{a}±1.53	88.10^{a}±0.42
LP+C	0.1045ab±0.001	96.17^{a}±2.48	88.10^{a}±0.39
LP+X	0.1030^{abc}±0.002	96.37^{a}±0.85	88.35^{a}±0.80
LP+C+X	0.1072^{a}±0.001	97.39^{a}±0.97	88.79^{a}±0.61
LF	0.0989^{c}±0.001	93.86^{ab}±1.31	87.46^{a}±0.31
LF+C	0.1013^{bc}±0.002	96.46^{a}±2.61	86.71^{a}±0.83
LF+X	0.1005^{bc}±0.001	93.98^{ab}±0.29	87.65^{a}±0.72
LF+C+X	0.1038^{ab}±0.003	96.47^{a}±0.94	87.08^{a}±0.71
SEM	0.001	0.500	0.183
P-Value	0.001	0.002	0.041

Values with different superscripts within a column differ significantly (p<0.05)

L: A is the lactic acid to acetic acid ratio; C-cellulase; X xylanase; C+X is the combinations of cellulase and xylanase; LP is the *Lactiplantibacillus plantarum*; LF is the *Limosilactobacillus fermentum*

Changes in pH and microbial count after aerobic exposure of maize silage

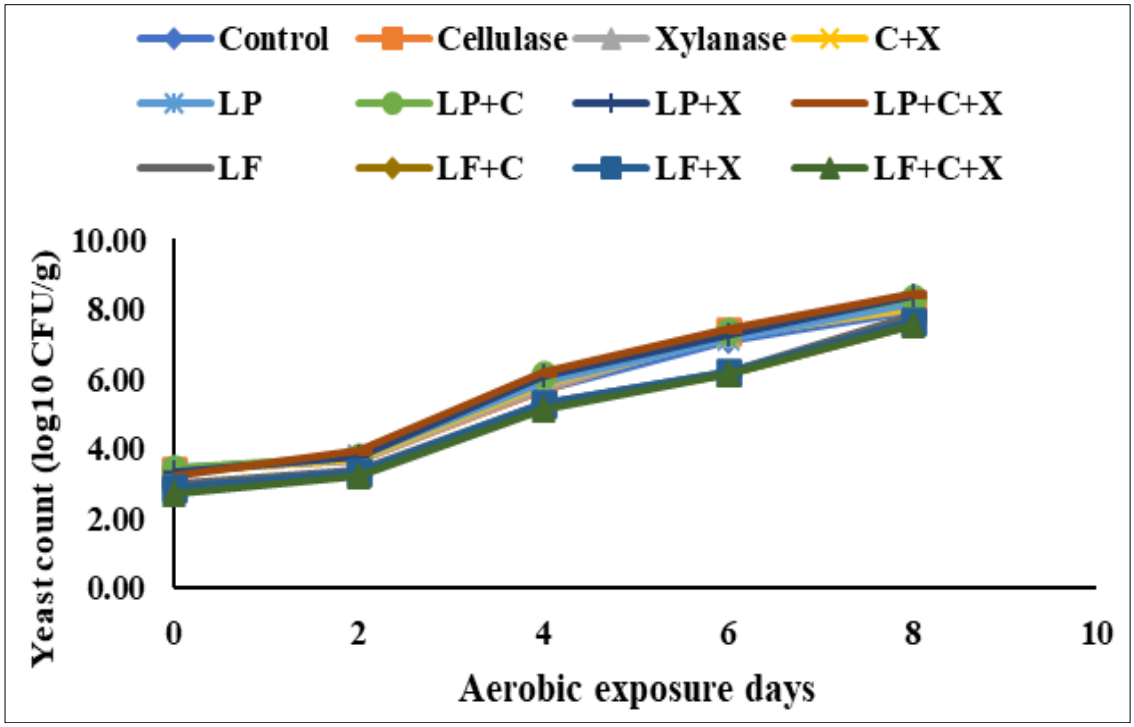
Table 4 shows the pH values of additive-treated maize silage at different aerobic exposure days (Day 0, 2nd, 4th, 6th and 8th). Initially, all silages had low pH values on day 0, but pH increased significantly (p<0.05) with each subsequent day of aerobic exposure. Throughout the 4th, 6th and 8th days of exposure, silages treated with heterofermentative bacterial inoculants maintained significantly (p<0.05) lower pH values compared to those treated with homofermentative bacterial inoculants. The lowest mean pH value (4.80) across different aerobic exposure days was observed in silage treated with LF+C+X, followed by LF+ (4.84) and LF+X (4.85) treated silages.

Figure 2 illustrates the yeast counts in additive-treated maize silage during aerobic exposure. Silage inoculated with LF and respective combinations exhibited the lowest yeast counts, correlating with higher aerobic stability. Yeast growth was significantly higher (p<0.05) in LP-treated silage, followed by the control and LF-inoculated silage. Figure 3 presents the mould counts (log10CFU/g) of additive-treated maize silage over different aerobic exposure days.

Table 4: Effect of additives and combinations on pH of maize silage on various aerobic exposure days

Treatment	Aerobic exposure days					Mean	SEM	Significance		
	0	2	4	6	8			P	T	P×T
Control	4.16	4.49	5.53	5.71	6.17	5.21^{de}	0.039	0.01	0.01	0.001
C	4.06	4.45	5.58	5.78	6.22	5.22^{de}				
X	4.05	4.42	5.53	5.76	6.19	5.19e				
C+X	4.08	4.47	5.62	5.81	6.31	5.26^{cd}				
LP	4.04	4.59	5.63	5.84	6.31	5.28^{c}				
LP+C	4.07	4.66	5.685	5.89	6.37	5.34^{ab}				
LP+X	4.04	4.61	5.66	5.86	6.33	5.30^{bc}				
LP+C+X	4.02	4.71	5.705	5.93	6.45	5.36^{a}				
LF	4.09	4.39	5.02	5.26	5.74	4.90^{f}				
LF+C	4.07	4.33	4.93	5.21	5.64	4.84^{gh}				
LF+X	4.02	4.34	4.98	5.23	5.7	4.85^{fg}				
LF+C+X	4.02	4.31	4.88	5.20	5.59	4.80^{h}				
Mean	4.06E	4.48^{D}	5.40^{C}	5.62^{B}	6.08^{A}					

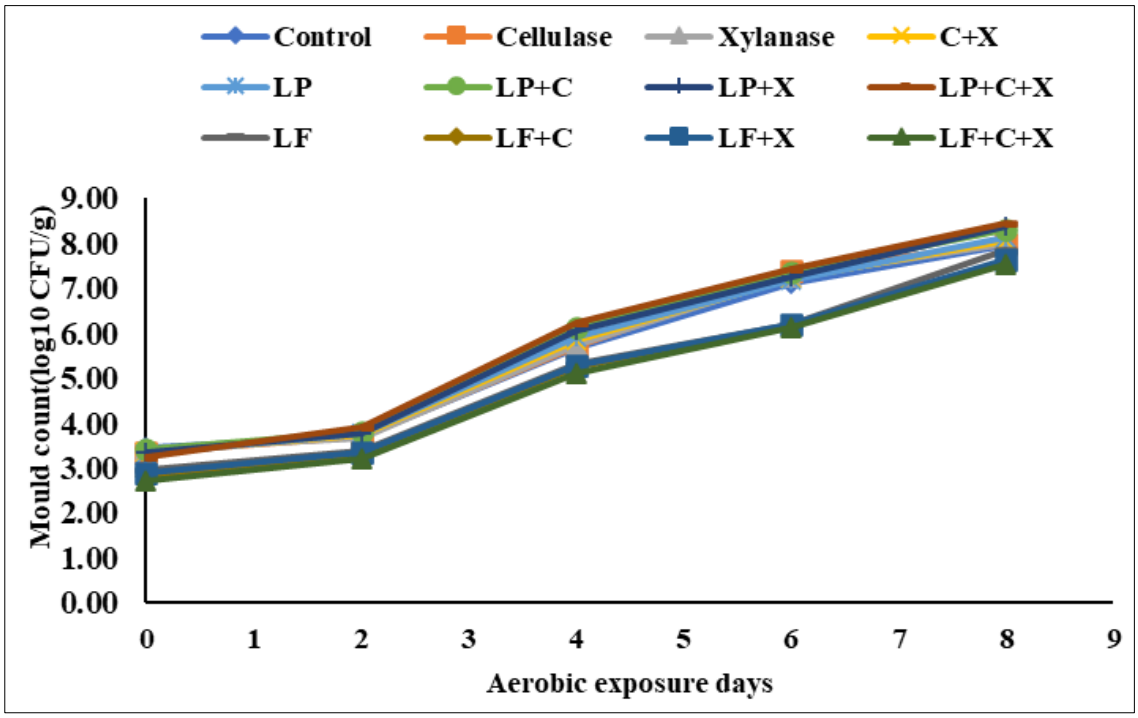
P is aerobic exposure period; T is the treatment; P×T is the interaction of period and treatment; *p<0.05 significant; SEM is the standard error of the means; a-h Values with distinct small letters indicate statistically significant variations between treatment in the same aerobic exposure days (p<0.05); A-E Significant variations aerobic exposure days in the same treatment are shown by values with distinct capital letters (p<0.05).



Values with different superscripts within a column differ significantly (p<0.05)

Fig 2: Effect of additives and aerobic exposure days on yeast count (log10CFU/g) of maize silage

L: A is the lactic acid to acetic acid ratio; C-cellulase; X xylanase; C+X is the combinations of cellulase and xylanase; LP is the *Lactiplantibacillus plantarum*; LF is the *Limosilactobacillus fermentum*



Values with different superscripts within a column differ significantly (p<0.05

Fig 3: Effect of additives and aerobic exposure days on mould count (log10 CFU/g) of maize silage

L: A is the lactic acid to acetic acid ratio; C-cellulase; X xylanase; C+X is the combinations of cellulase and xylanase; LP is the *Lactiplantibacillus plantarum*; LF is the *Limosilactobacillus fermentum*

Discussion

Fresh maize fodder exhibited suitable dry matter (30-35%) and water-soluble carbohydrate content (6-12%) recommended for ensiling, as noted by Tyrolová *et al.* (2017). Johansson (2011) [23] suggests that a dry matter content of less than 30% increases the risk of bacterial and fungal spoilage. The chemical composition of the maize fodder aligns with findings from previous studies (Arriola *et al.*, 2011) [4]. The pH and water-soluble carbohydrate levels observed were consistent with reported ranges for maize fodder.

The epiphytic LAB count in the maize fodder (5.99 log10 CFU/g) aligns with findings by Contreras-Govea *et al*. (2013). In contrast to our results, Hrafner *et al*. (2015) reported higher counts of epiphytic lactic acid bacteria, specifically 7.04 log10 CFU/g and yeast counts of 5.77 log10 CFU/g. Addah *et al*. (2011) [2] observed 8.57 log10 CFU/g of Lactobacillus counts in corn silage. According to Lin *et al*. (1992), the abundance of epiphytic LABs on fresh plants varies widely, ranging from less than 10 CFU/g to 10^4 CFU/g, influenced by factors such as crop species, climate, maturity stage and chopping method.

The results showing lower pH and increased lactic acid concentration in inoculated silages are consistent with previous studies, investigating bacterial inoculants and cellulase enzyme in sorghum forage silage, observed similar effects with reduced pH and higher lactic acid content compared to the control. Similarly, Sucu and Filya (2006) [46] noted elevated lactic acid levels and lower pH in corn silage treated with additives. Acosta *et al*. (2012) [1] found that inoculating whole maize fodder at ensiling with a commercial additive containing both homo and hetero-fermentative lactic acid bacteria resulted in significantly improved fermentation quality, characterized by lower pH and increased lactic acid concentration compared to untreated silage.

The finding of higher concentrations of acetic acid and a lower ratio of lactic acid to acetic acid in silage inoculated with the homofermentative group heterofermentative bacteria. This observation aligns with previous studies. Hashemzadeh-Cigari *et al*. (2013) [12] found a higher lactic acid to acetic acid ratio in alfalfa silage treated with a combination of homofermentative and propionate-producing bacterial inoculants. Similarly, Kleinschmit and Kung (2006) [25] reported that silage treated with L. *buchneri* had increased acetic acid concentrations and a decreased lactic acid to acetic acid ratio compared to untreated silage. This effect is likely due to the conversion of lactic acid to acetic acid, along with the production of 1, 2-propanediol and small amounts of ethanol by heterofermentative bacteria. According to Kung and Shaver (2001), an ideal lactic acid to acetic acid ratio should not be less than 3:1, with a higher ratio being preferable.

These finding are consistent with Cai *et al*. (1999) [7] and Kumari *et al*. (2023) [27], who concluded that inoculating silage with homofermentative lactic acid bacteria (LAB) has beneficial effects by promoting LAB growth. In the present study, LF inoculants were more effective in reducing yeast growth compared to LP, likely due to their higher acetate production. Acetic acid is known to inhibit yeasts responsible for aerobic spoilage, possibly due to its antifungal properties. Additionally, Danner *et al*. (2003) [13] identified that acetate exhibits antimicrobial properties against undesirable microbes. The reduction in yeast population by chemical additives may occur through mechanisms such as altering membrane functions or inducing cytosolic acidification.

The modified fitness value is influenced by pH, dry matter loss and ammonia nitrogen content of the silage. The modified fitness value is indicative of the efficacy of silage additives. The flieg point is determined by the pH and dry matter content of the silage. Kilic (1986) [24] introduced the rapid assessment method (flieg points) for quality evaluation based on dry matter and pH. According to this method, silage is categorized as very high quality if it scores between 81 and 100 on the flieg point scale. Based on flieg point assessment, all the silages in this study were classified as very good quality silage.

Additives used in this study effectively increased dry matter recovery. Treatment with inoculants containing LF led to lower dry matter recovery (DMR). This could be attributed to more extensive heterolactic fermentation and increased CO_2 production. Heterolactic bacteria convert lactic acid and carbohydrates into acetic and propionic acids, which contributes to dry matter losses characterized by CO_2 production (Filya, 2003) [19]. Similarly, studies by Arriola *et al*. (2021) [5] have reported that inoculants containing L. *buchneri* resulted in lower DMR compared to untreated silage. Typically, these losses range between 2% and 4% the predominant bacterial species and fermentable substrates play crucial roles in fermentation-related dry matter losses. Lactic acid bacteria (LAB) utilizing homofermentative pathways primarily produce lactate with minimal dry matter loss, whereas those employing heterofermentative pathways generate 1 mole of carbon dioxide per mole of glucose, resulting in losses of 2% to 4%.

Silage deterioration can be assessed by changes in pH and increases in yeast and mould populations. A lower pH following aerobic exposure indicates greater aerobic stability, suggesting reduced growth of spoilage bacteria. However, as aerobic exposure time lengthens, silage pH tends to rise. This can be attributed to the growth of various yeast species that degrade lactic acid into CO_2 and H_2O under aerobic conditions. This degradation of lactic acid contributes to an increase in silage pH, which further facilitates the growth of other spoilage organisms (McDonald *et al*., 1991) [30].

Silage treated with homofermentative bacterial inoculants exhibited lower aerobic stability compared to both the control and silage treated with heterofermentative bacteria. These findings are consistent with those of Filya and Succu (2007) [20], which could be attributed to homofermentative bacteria-treated silage containing higher levels of lactic acid and lower levels of acetic acid compared to silage treated with heterofermentative bacterial inoculants. Yeasts and moulds utilize lactic acid as a substrate, converting it into CO_2 and other byproducts, which accelerates the increase in silage pH.

Yeasts are commonly recognized as the primary initiators of aerobic deterioration in silage. The reduced aerobic stability observed in silages inoculated with LP may be attributed to higher yeast numbers. Additionally, LP inoculation often leads to lower levels of acetic acid production, which can accelerate yeast proliferation and diminish aerobic stability. According to Carvalho *et al*. (2015) [8], yeasts such as Candida spp., *Hansenula* spp., Pichia spp., Issatchenkia spp., and Saccharomyces spp. are primarily responsible for aerobic deterioration of silage by assimilating lactate. The aerobic stability of silage can be compromised if yeast counts exceed 6 log10 CFU/g of silage. In contrast, the use of heterofermentative lactic acid bacteria (LAB) during ensiling enhances acetate concentration, thereby improving aerobic stability (Danner *et al*., 2003; Filya & Sucu, 2007) [13, 20]. Consistent with previous studies, higher acetate levels in LF-inoculated silages resulted in lower yeast counts compared to LP-inoculated silages (Ranjit & Kung, 2000) [41].

The increased rate of deterioration observed in silages treated solely with homolactic acid-producing LAB is

attributed to the fermentation process that limits the accumulation of compounds such as acetic acid, which possesses antifungal properties. Undissociated acetic acid, along with other short-chain fatty acids, is effective in suppressing the growth of yeasts and moulds, whereas lactic acid alone is less effective against these organisms that initiate aerobic degradation (Danner *et al*., 2003 [13]. According to Chauhan *et al*. (2022), if the mould count reaches 7 log10 CFU/g of silage, it indicates reduced nutritional quality and signifies aerobic deterioration of the silage.

Conclusion

The additives effectively improved the maize silage fermentation parameters and quality attributes after aerobic exposure. Among the treatments, combination of LP+C+X was effective in improving the fermentation quality of silage, while the combination of LF and their respective has shown potential in maintaining quality of maize silage in terms of pH and yeast and mould count after aerobic exposure.

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