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Impact of individual and combined inoculants on physical and microbial attributes of silage

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Abstract
The present investigation was conducted at the Department of Animal Husbandry and Dairy science, College of Agriculture, Latur to know the physical and microbial qualities of silage with different inoculants by taking various treatment with different concentration of inoculants for preparation of silage. In this experiment, 6 treatments and 3 replications were laid out in a completely randomized design (CRD). All treatments with same oat-to-maize ratios were studied, with inoculation of *Lactobacillus plantarum* (I_1) and *Enterococcus faecium* (I_2). It was carried out to assess the effect of microbial inoculants on the physical and microbial properties of silage prepared from a 40% Oat and 60% Maize combination. Six treatments were formulated such as T_1 (control), T_2 (*Lactobacillus plantarum* 100%), T_3 (*Enterococcus faecium* 100%), T_4 (I_1) 50% + (I_2) 50%, T5 (I_1) 25% + (I_2) 75% and T6 (I_1) 75% + (I_2) 25%. Silage samples were fermented and evaluated after 45 and 90 days. The physical composition was assessed through sensory evaluation of silage. The Organoleptic test was carried out by including colour, odour and texture by penalties by using senses such as sight, odour, and touch. Microbial qualities results shows that bacterial population peaked at 45 days and decreased overtime, while the fungal population varied across treatments and time. The control (T_1) showed fungal presence initially, which disappeared by day 90. Treatments T_2, T_3, and T_5 showed reduced fungal growth over time, indicating partial control. T_4 maintained high fungal levels at both intervals, suggesting limited effectiveness of the 50:50 inoculant mix.

Keywords: Silage, lactobacillus plantarum, enterococcus faecium, microbial inoculants, physical attributes, microbial quality, oat-maize combination, fermentation

Introduction
Forages serve as the main source of nutrients for dairy animals, making them an essential part of the dairy feed supply chain. Livestock are typically fed using three types of fodder i.e. fresh green forage, silage (preserved in anaerobic conditions), and hay (dried green fodder). The most commonly cultivated fodder crops include cereal varieties such as sorghum, maize, rye, and oat, along with legumes like lucerne, cowpea, and berseem. These forages are considered highly nutritious and economical for feeding dairy cattle. Therefore, enhancing the nutritional quality of forage crops is crucial for boosting animal productivity and ensuring the availability of high-quality animal-based food products (Chaudhary, 2008) [2]. Oats (*Avena sativa L.*) are one of the key winter fodder crops cultivated worldwide. In various parts of the world, oats are grown not only for grain but also for forage, fodder, hay, haylage, silage, and chaff. During periods of fodder shortage, oats are commonly used as silage due to their soft texture and high palatability, which makes them a preferred feed for livestock (Suttie and Reynolds, 2004) [9]. Maize (*Zea mays L.*) is extensively cultivated worldwide due to its adaptability to diverse environmental and agronomic conditions, consistent yield, high energy content, and excellent suitability for silage. The efficiency and quality of silage depend largely on the microbial dynamics during the ensiling process. To optimize fermentation and improve silage stability, various inoculants primarily strains of lactic acid bacteria (LAB) are now extensively employed. Commonly used inoculants include *Lactobacillus plantarum, Pediococcus pentosaceus, Lactobacillus buchneri, and Enterococcus faecium*, each contributing specific benefits to the silage ecosystem. *L. plantarum* and *P. pentosaceus* enhance rapid lactic acid production, thereby lowering pH quickly and minimizing proteolysis, while *L. buchneri* is known for improving aerobic

stability by producing acetic acid, which inhibits the growth of spoilage organisms such as yeasts and molds (Muck, 1998; Kung *et al*., [1]). Recent advancements have also introduced multi-strain and enzyme-enhanced inoculants, designed for specific forages such as maize, oats, and sorghum, to improve fiber degradation and overall feed digestibility.

Materials and Methods

Location

The experiment was carried out on the field of Department of Animal Husbandry and Dairy Science, College of Agriculture, Latur.

Fodder Collection and Preservation through Ensiling

The forage crops used for silage preparation were Maize (variety: African Tall) and Oat (variety: NDO-10)—were sourced from the fodder production unit of the Department of Animal Husbandry and Dairy Science, College of Agriculture, Latur. Maize was harvested at 80-90 days and oat at 90-100 days, specifically at the milk to dough stage, when approximately 50% of the grain's weight had developed—an ideal stage for silage making. After harvest, maize was field-wilted for 5-6 hours to lower its moisture content, while oat was allowed to wilt for 24 hours post-harvest to reduce moisture and enhance dry matter content. The harvested fodder was then chopped into 2 cm pieces using a chaff cutter, which facilitated compacting and helped eliminate air when packed into heavy-duty micron plastic bags for ensiling.

Procurement of inoculant

The bacterial cultures used in the study was *Lactobacillus plantarum* and *Enterococcus faecium* which were obtained from ICAR-NBAIM, Mau Nath Bhanjan, Uttar Pradesh, and subsequently cultured in the Department of Plant Pathology, College of Agriculture, Latur.

Experimental Details

A completely randomized design with three replicates was used the treatment details and are as follows

Treatment Details:

Sr.	Treatment	Contents
1.	T_1	40% Oat + 60% Maize (Control)
2.	T_2	40% Oat + 60% Maize (I_1) 100%
3.	T_3	40% Oat + 60% Maize (I_2) 100%
4.	T_4	40% Oat + 60% Maize (I_1) 50% + (I_2) 50%
5.	T_5	40% Oat + 60% Maize (I_1) 25% + (I_2) 75%
6.	T_6	40% Oat + 60% Maize (I_1) 75% + (I_2) 25%

Statistical Analysis

The data generated during the experimental period were subjected to statistical analysis of variance (ANOVA) by completely randomized design as described in Panse and Sukatme, 1967 [7] with three replications. The results of statistical analysis with significant differences between treatments were further followed by WASP-Web Agri Stat Package.

Silage preparation

Flowchart for Preparation of silage

Crop Selection and Harvesting

- Maize harvested at 55–70 days
- Oat harvested at 90–100 days
- Both at milk to dough stage (≈ 50% grain maturity)

⬇

Pre-Wilting / Drying

- Maize: Shade-dried for 5–6 hours to reduce moisture to 65–70%
- Oat: Wilted for 24 hours to reduce moisture and increase dry matter content

⬇

Chopping

- Fodder chopped into 2 cm pieces using a chaff cutter to facilitate compaction further divided into six treatment groups.

⬇

Application of Inoculants

Lactobacillus plantarum and *Enterococcus faecium* applied to treatments T_1 to T_6

- Concentration: 1×10^7 CFU/g of fodder.
- Thorough mixing to ensure even distribution.

⬇

Packing and Ensiling

- Treated fodder packed tightly into plastic bags by removing air completely to maintain anaerobic conditions.

⬇

Fermentation

- Bags stored at room temperature.
- Sampling and bag opening at 45 and 90 days intervals

Physical Quality of Silage

Physical attributes such as colour, odour, and texture were evaluated through visual observation. An organoleptic test was conducted, which included the assessment of these qualities using a structured questionnaire. The evaluation relied on the senses of sight, smell, and touch to identify specific characteristics, while the panel was restricted to individuals who had undergone training in forage, silage production, and feed technology.

Colour of silage

Colour of silage samples are determined with the help of munsell colour chart.

Odour of silage

Odour of samples is determined by sensory evaluation.

Texture of silage
The texture of silage was examined through physical evaluation, taking into account the softness of leaves and stems, its resistance to pressure when squeezed tightly in the hand, and its ability to fragment into smaller pieces, which together indicated overall textural quality.

Microbial Quality of silage
Silage samples were examined after 45 and 90 days of fermentation to quantify bacterial and fungal populations. Microbial enumeration was performed using the serial dilution and pour plate method, with Nutrient Agar (NA) employed for bacterial counts and Potato Dextrose Agar (PDA) for fungal counts.

Results and Discussion
Physical Qualities of Silage
Colour

Table 4.1: Colour of Silages prepared from different inoculants and its combinations

Treatment	Colour	
	45 Days	90 Days
T_1 - 40% Oat + 60% Maize Control	Slightly Brown	Dull Brown
T_2 - 40% Oat + 60% Maize (I_1) 100%	Yellowish Green	Light Yellow
T_3 - 40% Oat + 60% Maize (I_2) 100%	Mild Green	Dark Brown
T_4 - 40% Oat + 60% Maize (I_1) 50% + (I_2) 50%	Yellow Brown	Dark Brown
T_5 - 40% Oat + 60% Maize (I_1) 25% + (I_2) 75%	Yellow Brown	Olive Brown
T_6 - 40% Oat+ 60% Maize (I_1) 75% + (I_2) 25%	Yellow Green	Light Brown

As indicated in the Table 4.1; The colour of silage reflects fermentation quality and pigment preservation. At 45 days, the T_1 (Control) appeared slightly brown, indicating moderate fermentation. In contrast, T_2 showed a yellowish green colour, suggesting better pigment retention and effective fermentation. T_3 appeared mild green, while combinations of I_1 and I_2 (T_4-T_6) ranged from yellow brown to yellow green, showing intermediate results. By 90 days, colour darkened in all treatments. The T_1(Control) turned dull brown, while T_2 maintained a light yellow, indicating superior preservation with I_1. Treatments with I_2 or its combinations (T_3-T_5) turned dark to olive brown, showing lower stability. T_6 retained a light brown tone, further supporting the effectiveness of I_1.
These results are in line with the findings of Markos, F. D. (2015) [4], who observed that the silage produced using a combination of 50% Hybrid Napier, 50% maize, and 3% molasses displayed a light-green to brown color. Similarly, Jadhav, K. G. (2019) [3] reported an olive green color in maize silage treated with various LAB strains as well as in the control across 10, 20, 30, and 45 days of ensiling.

Odour

Table 4.2: Odour of Silages prepared from different inoculants and its combinations

Treatment	Odour	
	45 Days	90 Days
T_1 - 40% Oat + 60% Maize Control	Strong Alcoholic	Alcoholic
T_2 - 40% Oat + 60% Maize (I_1) 100%	Slightly Alcoholic	Fruity
T_3 - 40% Oat + 60% Maize (I_2) 100%	Pleasant Alcoholic	Mild Grassy
T_4 - 40% Oat + 60% Maize (I_1) 50% + (I_2) 50%	Slightly Alcoholic	Faint Fruity
T_5 - 40% Oat + 60% Maize (I_1) 25% + (I_2) 75%	Slightly Alcoholic	Fruity
T_6 - 40% Oat+ 60% Maize (I_1) 75% + (I_2) 25%	Pleasant Alcoholic	Mild Fruity

From the table 4.2; observed that odour is a key sensory indicator of silage quality, reflecting the type and extent of fermentation. At 45 days, the T_1 (Control) emitted a strong alcoholic smell, indicating undesirable fermentation and potential nutrient loss. In contrast, inoculated treatments showed milder and more pleasant odours. Treatments T_2 - (I_1) 100% and T_4 - T_6 (I_1+I_2) combinations had slightly alcoholic odours, suggesting better-controlled fermentation. Treatment T_3 - I_2 (100%) and T_6 - (I_1) 75% + (I_2) 25% produced a pleasant alcoholic aroma, indicating balanced fermentation and effective microbial activity.
By 90 days, improvements in odour were observed in all inoculated treatments. The control (T_1) remained alcoholic, showing poor fermentation stability. T_2 and T_5 developed a fruity odour, reflecting desirable lactic acid fermentation. T_3 had a mild grassy smell, while T_4 and T_6 showed faint to mild fruity notes. These odours suggest improved fermentation quality and reduced production of unwanted compounds.
These findings are in agreement with Markos, F. D. (2015), who reported that silage made from a blend of 50% HN + 50% maize + 3% molasses, as well as 50% maize + 50% lucerne, had a pleasant alcoholic smell.

Texture

Table 4.3: Texture of Silages prepared from different inoculants and its combinations

Treatment	Texture	
	45 Days	90 Days
T_1 - 40% Oat + 60% Maize Control	Slightly Firm	Slightly Soft
T_2 - 40% Oat + 60% Maize (I_1) 100%	Slightly Firm	Slightly Soft
T_3 - 40% Oat + 60% Maize (I_2) 100%	Slightly Soft	Slightly Soft
T_4 - 40% Oat + 60% Maize (I_1) 50% + (I_2) 50%	Slightly Firm	Slightly Moist
T_5 - 40% Oat + 60% Maize (I_1) 25% + (I_2) 75%	Slightly Sticky	Slightly Slimy
T_6 - 40% Oat+ 60% Maize (I_1) 75% + (I_2) 25%	Slightly Firm	Slightly Soft

From the below table 4.3; At 45 days, the control (T_1) and most inoculated treatments (T_2, T_4, and T_6) exhibited a slightly firm texture, indicating good compaction and moderate moisture retention. T_3 (I_2) 100% showed a slightly soft texture, suggesting higher moisture or less compact structure. T_5 (I_1) 25% + (I_2) 75% was slightly sticky, which may indicate excessive moisture or early-stage spoilage tendencies. By 90 days, the texture of most treatments transitioned toward softer or moister forms. The control (T_1) and several others (T_2, T_3, and T_6) became slightly soft, reflecting natural softening over time. T_4 was slightly moist, indicating balanced fermentation with adequate moisture preservation. However, T_5 developed a slightly slimy texture, suggesting undesirable microbial activity and poorer fermentation quality. With comparison of control treatment T_1 (40% Oat + 60% Maize), inclusion of oat in silage preparation with maize in treatments composition (T_2, T_3 ,T_4, T_5, and T_6) helps to change texture from slightly firm to soft, soft to very soft at 45 and 90 days of ensiling.

These results are in agreement with the observations of Markos, F. D. (2015) [4], who reported that the texture of silage prepared with a combination of 50% HN + 50% maize + 3% molasses and 50% maize + 50% lucerne was firm.

Microbial analysis of silage prepared from different inoculants and its combinations

a) Population of Bacteria

Table 4.4: Population of Bacteria in silage prepared from Maize and Oat combination

Treatment	Population of Bacteria (10^7)	
	45 Days	60 Days
T_1 - 40% Oat + 60% Maize Control	32.66	30.66
T_2 - 40% Oat + 60% Maize (I_1) 100%	36.00	37.33
T_3 - 40% Oat + 60% Maize (I_2) 100%	44.33	43.33
T_4 - 40% Oat + 60% Maize (I_1) 50% + (I_2) 50%	43.66	34.66
T_5 - 40% Oat + 60% Maize (I_1) 25% + (I_2) 75%	46.05	41.00
T_6 - 40% Oat+ 60% Maize (I_1) 75% + (I_2) 25%	47.00	45.00
Mean	41.62	38.66
SEM ±	1.192	1.209
CD 5%	3.675	3.726

Table 4.4.1: Analysis of variance for Bacterial population

ANOVA for Population of Bacteria (10^7) at 45 days						
Source	**DF**	**SS**	**MS**	**F**	**Result**	**P-value**
Treatments	5	515.7886	103.1577	24.16655	**	0.00001
Error	12	51.2234	4.268617			
Total	17	567.012				
ANOVA for Population of Bacteria (10^7) at 90 days						
Source	**DF**	**SS**	**MS**	**F**	**Result**	**P-value**
Treatments	5	447.3333	89.46667	20.38481	**	0.00002
Error	12	52.66667	4.388889			
Total	17	500				

The bacterial population in the ensiled mixture of 40% oat and 60% maize exhibited highly significant differences ($p<0.01$) among treatments at both 45 and 90 days, as confirmed by ANOVA analysis. From the table 4.4, ANOVA table 4.4.1

At 45 days, the bacterial count ranged from 32.66 × 10^7 CFU/g T1(Control) to 47.00 × 10^7 CFU/g T6 - (I1) 75% + (I2) 25%. Treatments inoculated with bacterial cultures demonstrated markedly higher microbial counts compared to the control. Notably, T6 and T5 recorded the highest populations, indicating enhanced microbial activity and likely efficient fermentation. In contrast, the lowest population in T_1 reflected the absence of microbial inoculants. The F-value of 24.17, with a CD at 5% of 3.675, confirms that the observed variations were statistically significant ($p = 0.00001$).

At 90 days, a similar trend continued, with the bacterial population ranging from 30.66 × 10^7 CFU/g (T_1) to 45.00 × 10^7 CFU/g (T_6). Inoculated treatments, particularly T_6, T_3 (I2 100%), and T_5, retained significantly higher bacterial counts, suggesting sustained microbial viability over time. The F-value of 20.38 and CD at 5% of 3.726 again affirm the highly significant differences ($p=0.00002$) among treatments. Overall, inoculated treatments significantly improved the bacterial population compared to the control. Treatments with I_2 either alone (T_3) or in combination with I_1 (T_5, T_6) showed the greatest enhancement, likely due to the synergistic effect or higher adaptability of the bacterial strains. These results imply that bacterial inoculation promotes active fermentation, which is crucial for better nutrient preservation, rapid pH decline, and improved silage quality.

These results are consistent with those of Arasu *et al*. (2014) [1], who reported bacterial populations of 53.10 × 10^7 cfu/g in rye silage and 16.18 × 10^7 cfu/g in Italian ryegrass (IRG) silage, indicating variation based on the type of forage used. Similarly, Ravindra S. N. (2011) [8] found that silage treated with *Lactobacillus plantarum* had a bacterial population of 35.7 × 10^6 cfu/g.

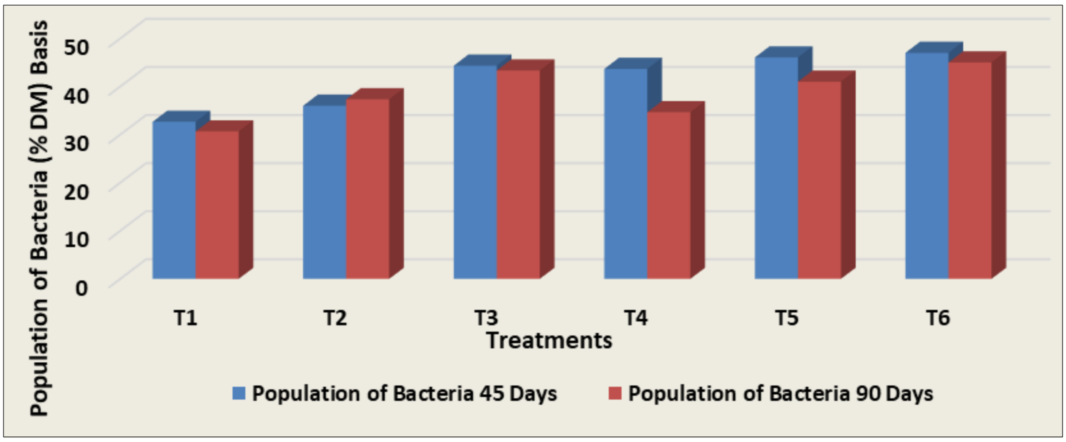

Fig 1: Population of Bacteria in Silage prepared from different Inoculants & its combination

b) Population of Fungi

Table 4.5: Population of Fungi in silage prepared from Maize and Oat Combination

Treatment	Population of Fungi (10^4)	
	45 Days	90 Days
T_1 - 40% Oat + 60% Maize Control	0.66	0.00
T_2 - 40% Oat + 60% Maize (I_1) 100%	1.00	0.33
T_3 - 40% Oat + 60% Maize (I_2) 100%	1.00	0.33
T_4 - 40% Oat + 60% Maize (I_1) 50% + (I_2) 50%	1.00	1.00
T_5 - 40% Oat + 60% Maize (I_1) 25% + (I_2) 75%	1.00	0.33
T_6 - 40% Oat+ 60% Maize (I_1) 75% + (I_2) 25%	0.00	0.00
Mean	0.77	0.33
SEM ±	0.136	0.124
CD 5%	0.419	0.3812

Table 4.5.1: Analysis of variance for Fungal population

ANOVA of Population of Fungi (10^4) at 45 days						
Source	**DF**	**SS**	**MS**	**F**	**Result**	**P-value**
Treatments	5	2.444444	0.488889	8.8	**	0.00105
Error	12	0.666667	0.055556			
Total	17	3.111111				
ANOVA of Population of Fungi (10^4) at 90 days						
Source	**DF**	**SS**	**MS**	**F**	**Result**	**P-value**
Treatments	5	2.000044	0.400009	7.20016	**	0.00249
Error	12	0.666667	0.055556			
Total	17	2.666711				

* P<0.05; ** P<0.01; NS - non significant

As shown in Table 4.5, ANOVA table 4.5.1 At 45 days, fungal counts varied between 0.00 × 10^4 CFU/g [T_6 (I_1) 75% + (I_2) 25%] and 1.00 × 10^4 CFU/g in several treatments including T_2 - (I_1) 100%, T_3 - (I_2) 100%, T_4 - (I_1) 50% + I_2 50%, and T_5 - (I_1) 25% + (I_2) 75%. The control (T_1) showed a moderate count of 0.66 × 10^4 CFU/g, whereas the complete absence of fungal growth in T_6 suggests enhanced antifungal activity, likely due to competitive inhibition by beneficial bacteria or improved fermentation conditions. The F-value of 8.80 and CD at 5% of 0.419 confirm the statistical significance of the observed differences (p=0.00105).

By 90 days, overall fungal counts declined, with T_1 and T_6 showing complete absence of fungal population, and most other treatments (T_2, T_3, T_5) showing a reduced count of 0.33 × 10^4 CFU/g. Notably, T_4 retained the highest fungal count (1.00 × 10^4 CFU/g), possibly due to suboptimal microbial dominance or slower acidification. The ANOVA results with an F-value of 7.20 and CD at 5% of 0.3812 again highlight significant treatment effects (p = 0.00249).

These findings suggest that fungal growth was effectively inhibited in silage treated with a higher proportion of inoculant I_1 (as in T_6), likely due to improved fermentation dynamics and rapid pH reduction. The overall lower fungal counts at 60 days further reflect increased stability of silage with time and the efficacy of microbial inoculants in controlling spoilage organisms, enhancing the hygienic and preservational quality of the silage.

Similar findings were reported by Arasu *et al.* (2014) [1], who observed fungal populations in rye grass silage, with the control treatment showing a count of 0.04 × 10^2 cfu/g and the silage treated with *Lactobacillus plantarum* inoculant showing a reduced count of 0.02 × 10^2 cfu/g. The application of lactic acid bacteria in silage is generally aimed at suppressing the growth of undesirable microorganisms such as wild yeasts, fungi, and actinomycetes, thereby enhancing silage stability upon aerobic exposure (Weinberg and Muck, 1996) [10]. Additionally, O'Brien *et al*. (2005) [6] documented fungal contamination in baled grass silage in the Irish midlands, noting that 90% of bales exhibited fungal presence, with an average of 5% of each bale's surface affected.

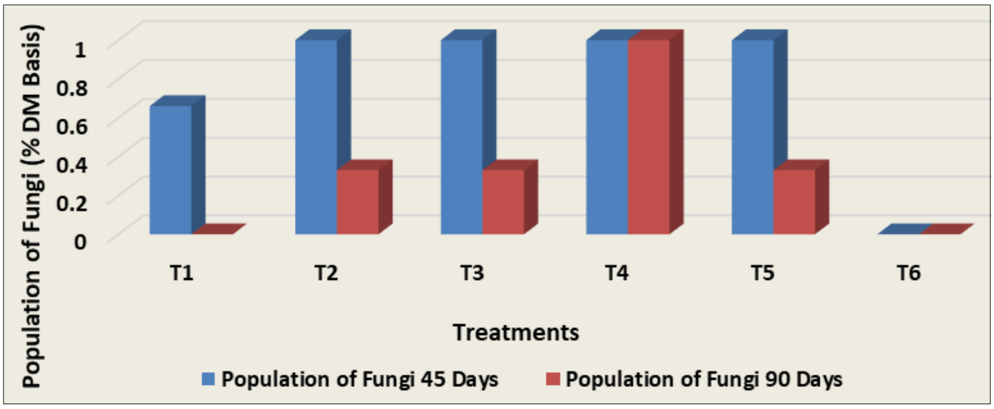

Fig 2: Population of Fungi in Silage prepared from different Inoculants & its combination

Conclusion

The evaluation of physical characteristics revealed that inoculated silages consistently outperformed the control in terms of colour, odour, and texture. The control treatment (T_1) showed signs of oxidative deterioration, unpleasant odour, and poor compaction, all of which are indicators of inferior fermentation and reduced silage quality. In contrast, inoculated treatments, particularly T_2 (*Lactobacillus plantarum* 100%) and T_6 (75% *Lactobacillus plantarum* + 25% *Enterococcus faecium)*, retained desirable greenish hues, developed pleasant fruity-sweet odours, and maintained firm, compact textures, signifying effective fermentation, minimal spoilage, and enhanced palatability. Treatments T_4 and T_5 displayed intermediate quality, while T_3 (*Enterococcus faecium* 100%) remained less effective compared to other inoculated combinations. Overall, the results confirm that the use of appropriate inoculants, especially *Lactobacillus plantarum* either alone or in higher proportions, plays a pivotal role in preserving pigment, promoting favorable fermentation, and ensuring better physical attributes of oat-maize silage.

The microbial assessment revealed that inoculated silages supported better bacterial growth while effectively suppressing fungal activity compared to the control. Among all treatments, T_6 (75% *Lactobacillus plantarum* + 25% *Enterococcus faecium*) consistently exhibited the highest bacterial population coupled with complete inhibition of fungi, highlighting its strong fermentative and antifungal efficiency. T_5 also performed well, though less effectively than T_6, while T_4 showed poor fungal control despite moderate bacterial activity. Overall, mixed inoculants, particularly those dominated by *Lactobacillus plantarum*, proved most effective in enhancing microbial balance, ensuring efficient fermentation, and improving silage stability and quality.

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