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VARIATION OF EPIPHYTIC FLORA AFFECTING SILAGE QUALITY IN PURE AND MIXED MUNG BEAN AND SWEET SORGHUM

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Abstract: This study was carried out to determine the microorganism population affecting silage quality of sweet sorghum and mung bean cultivated with different sowing patterns as mixture or sole crop. Twin row (20×55 cm row spacing), narrow row (55 cm row spacing) and conventional row (75 cm row spacing) were used as mixture sowing patterns. The mixtures were formed based on the plant densities and alternative row numbers of sweet sorghum and mung bean. Sowing was made on alternation rows of 1 row mung bean plus 1 row sweet sorghum (R1:1) and 1 row mung bean plus 2 rows sweet sorghum (R1:2). In pure and mixed cultivations, the plant density of sorghum was 14 plants m^{-2} while the plant densities of mung bean were 14, 21 and 28 plants m^{-2}. The experiment was planned as two-factors (sowing patterns and mixtures) and was arranged in randomized blocks according to the split plot design with 3 replications. Pure and mixed plants were harvested when the sweet sorghum plant reached the dough stage. Lactic acid bacteria, enterobacteria and yeast and mold populations in the plant epiphytic flora were investigated under experimental factors. There were significant effects of the main factors and their interactions on the plant epiphytic microorganisms. According to the results obtained from the current study, mixed cultivation of sweet sorghum and mung bean in conventional row pattern improved the desired lactic acid bacteria population for silage quality, while reducing the undesirable enterobacteria and yeast and mold population for silage quality. It was determined that the (R1:2) MB14+SS14 mixed cultivation system was the most suitable mixture in conventional row pattern in terms of high lactic acid bacteria population.

Keywords: Sowing patterns, Intercropping, Lactic acid bacteria, Mung bean, Silage, Sweet sorghum

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1. Introduction

The product formed as a result of fermentation of forage plants in an oxygen-free environment is silage (Kizilsimsek et al., 2017). The most important advantage of silage production is that it produces a stable feed containing high energy and digestible nutrients by providing a high dry matter conservation compared to dry forages (Ertekin et al., 2022). Silage making has many advantages over other roughage storage methods. For example, nutrient loss in dry storage can be higher than silage (Ertekin and Kızılşimşek, 2020). However, the factors affecting the fermentation quality in silage production depend on the chemical composition and dry matter content of the ensiled plants. In addition to these features, the epiphytic (natural) flora of the plants entering the silo is an important factor (Kung et al., 2018). Microorganisms found in this epiphytic flora are divided into desirable and undesirable microorganisms (Kung, 2010). Lactic acid bacteria represent desirable microorganisms, while enterobacteria, yeast and molds are in the undesirable class (Santos et al., 2015). These microorganisms can cause a wide variety of end products to occur in the silo (Kung and Shaver, 2001).

Knowing the microbial population of the forage plant with sufficient chemical composition and dry matter content can help to obtain a healthy silage (Kung et al., 2018). For example, the insufficient lactic acid bacteria population of an ensiled forage plant may delay fermentation and increase nutrient loss in the silo (Kızılşimşek et al., 2016). It is a popular method to inoculate forage plants with lactic acid bacteria before ensiling when insufficient lactic acid bacteria population is detected in the natural flora of forage plants or when there is a high presence of undesirable microorganisms (Ertekin and Kızılşimşek, 2020). Therefore, it is of great importance to know the microbial population in the natural flora of the ensiled plants.

In this study, it was aimed to examine the microbial population in the epiphytic flora of sweet sorghum and mung beans grown with different sowing patterns and mixed growing systems and to facilitate the storage of forage by ensiling.

2. Material and Methods

2.1. Material

In this study, the ERDURMUŞ sweet sorghum cultivar registered by the Western Mediterranean Agricultural Research Institute Directorate in 2018 and the mung bean population obtained from Uzbekistan through a commercial company engaged in horticultural seeds were used as plant material.

2.2. Soil and Climatic Characteristics of the Experimental Field

This study was carried out at Hatay Mustafa Kemal University, Faculty of Agriculture, Field Crops Department, Telgaliş Research and Application Field (36°15'13.56"N 36°30'7.96"E, altitude 96 m) for two years in 2019 and 2020 under second crop production conditions. The soil of the experiment area is clay-loam and the total salt content is low and slightly alkaline. Lime and phosphorus content is moderate and organic carbon content is quite low. The climate data of the region were given in Figure 1. The rainfall values of the growing periods of 2019 and 2020 were considerably lower than the long-term averages. On the other hand, the opposite situation occurred in temperature values.

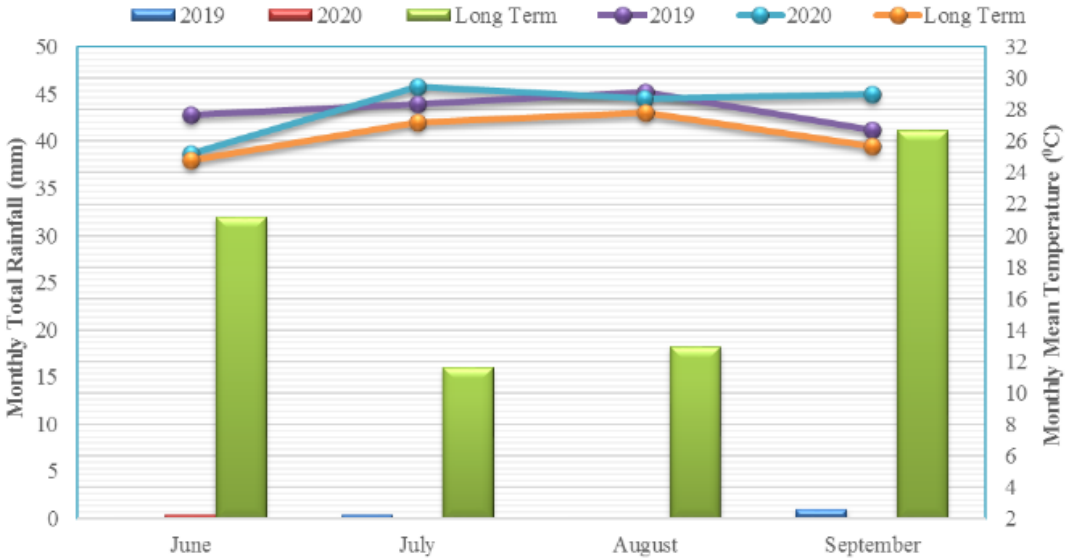

Figure 1. Some climatic data of the experimental field.

2.3. Cultivation Techniques, Experimental Factors and Harvest

The study was planned as two-factor and was carried out in randomized blocks according to the split plot design with three replications. The trial was established on June 20 in 2019 and on June 23 in 2020 under the second crop production conditions. The main factors of the experiment were sowing patterns and the sub-factors were mixed cultivation systems. Twin row (20×55 cm row spacing), narrow row (55 cm row spacing) and conventional row (75 cm row spacing) were used as sowing patterns (Figure 2).

Figure 2. An image taken with a drone from the trial area.

The mixtures were formed based on the plant densities and alternative row numbers of sweet sorghum and mung bean. Sowing was done on alternation rows of 1 row mung bean plus 1 row sweet sorghum (R1:1) and 1 row mung bean plus 2 rows sweet sorghum (R1:2). The plant density of sweet sorghum was included in the mixtures as 14 plants m^{-2} (SS14) and the plant densities of mung bean as 14 plants m^{-2} (MB14), 21 plants m^{-2} (MB21) and 28 plants m^{-2} (MB28).

In-row distances calculated according to sowing patterns of plant species were taken into account while sowing. Before planting, 5 kg da^{-1} NPK were applied and mixed into the soil basally. When the plants reached a height of 40-50 cm (approximately 30 days after emergence), a deep hoe was made by hand for weed control and soil aeration in the entire experimental area. In both years, 2 days after hoeing, 5 kg da^{-1} N as urea was applied and irrigated at field capacity. Harvest was done on September 20 in 2019, and on September 23 in 2020, about 90 days after emergence, when sweet sorghum plants reached the dough stage and mung bean plants reached 50% pod forming stage. Side rows and 0.5 m lengths from the beginning of each row were removed from all plots as a side effect and the plants were cut manually with the help of a sickle. The plant species harvested from the mixtures were weighed separately and their fresh weights were recorded. Fresh weight ratios of mixtures were calculated based on the fresh weights of the plant species obtained from the plots. 250 g samples were taken to determine the microbial

population and transported from the field to the laboratory by cold chain.

2.4. Method

According to Yan et al. (2019), 20 g of fresh sample from each treatment was homogeneously blended in 180 mL of sterile Ringer's solution for 60 seconds with the help of a blender (Arçelik K8130 MV). Then the obtained samples were filtered through Whatman no 54 filter paper. In the dilution series (from 10-1 to 10-10) made from these samples, Lactic acid bacteria (LAB), enterobacteria and yeast and mold populations were determined using the MRS (DE MAN, RAGOSA, SHARPE) agar, VRB-G (Violed Red Bile Glucose) and MEA (Malt Extract Agar), respectively (Figure 3).

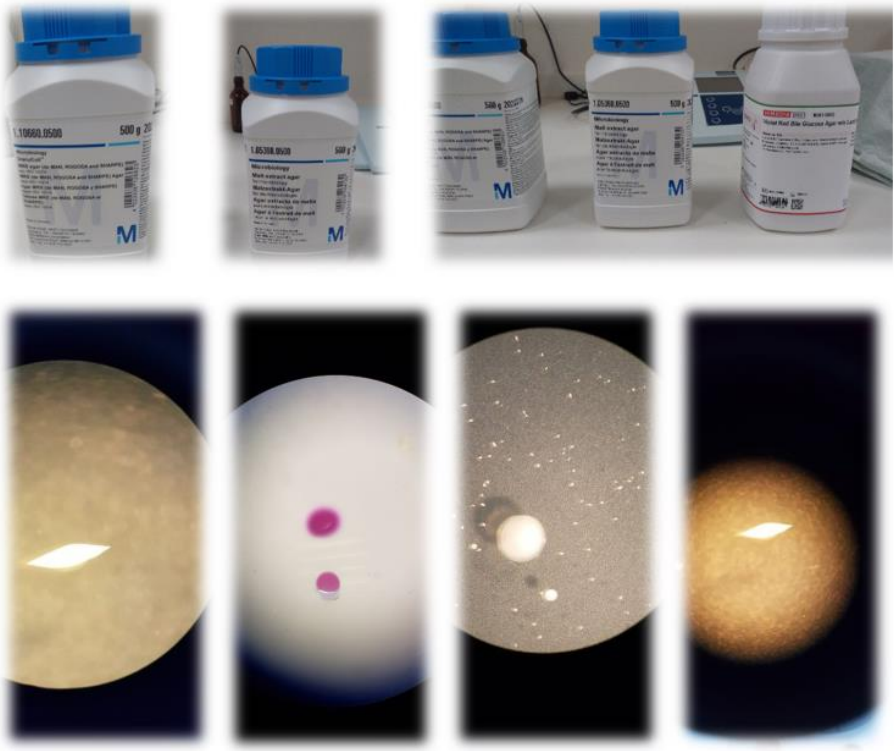

Figure 3. The media used to determine the microorganism population and the counted microorganism colonies.

For this purpose, the filtrates obtained from fresh materials according to a certain procedure were inoculated to agar media sterilized in an autoclave (WiseClave WAC-80) and kept in a water bath (WiseCircu WCB-22) under a sterile cabinet on agar media kept in a water bath (WiseCircu WCB-22). Pouring of the agar media and inoculating of the microorganism were made into disposable sterile plastic petri dishes. MRS and MEA media prepared to determine the LAB and yeast and mold numbers, respectively, were incubated in anaerobic conditions at 37 °C for 48 hours in a climate cabinet (Devpet Esde series). In addition, samples containing VRB-G prepared to determine the number of enterobacteria were incubated at 33 °C for 18 hours. A maximum of 300 colonies were counted in each petri dish.

2.5. Statistical Analysis

Microorganism population data obtained from present study were subjected to analysis of variance according to split-split plots in randomized block design with trial factors and year effect. As a result of variance analysis, microorganism populations that were found to be important ($P < 0.05$) statistically were grouped by Tukey pairwise test (Genç and Soysal, 2018).

3. Results and Discussion

In this study, lactic acid bacteria, enterobacteria and yeast and mold populations in the epiphytic flora of mung beans and sweet sorghum plants grown with different sowing patterns and intercropping systems and affecting silage quality were investigated. The effects of years, sowing patterns (SP), mixtures (M) and SP×M interaction on lactic acid bacteria population were found to be significant (Table 1). Lactic acid bacteria count results of the years, sowing patterns and mixtures were given in Table 1. In 2019 and 2020, lactic acid bacteria were 3.04 $\log_{10}$cfu g^{-1} DM and 2.96 $\log_{10}$cfu g^{-1} DM, respectively. The number of lactic acid bacteria was determined between 2.84 $\log_{10}$cfu g^{-1} DM and 3.30 $\log_{10}$cfu g^{-1} DM in sowing pattern treatments. The highest plant lactic acid bacteria were obtained from conventional row cultivation. The lowest plant lactic acid bacteria was obtained from twin row and this treatment was statistically in the same group with narrow row. The number of lactic acid bacteria in the mixtures varied between 2.03 $\log_{10}$cfu g^{-1} DM and 3.53 $\log_{10}$cfu g^{-1} DM. While the highest lactic acid bacteria were detected in the SS14 system, the lowest was obtained from the MB21 system.

According to interactions (SP×M), the number of plant lactic acid bacteria varied between 1.80 $\log_{10}$cfu g^{-1} DM

and 4.03 log_{10}cfu g^{-1} DM (Figure 4). The highest lactic acid bacteria count was detected in the (R1:2) MB14+SS14 mixed system of conventional row cultivation. The lowest lactic acid bacteria were obtained from MB21 treatment of twin row. The lactic acid bacteria population obtained from the current study was lower in pure mung bean cultivation compared to pure sweet sorghum cultivation. A very high lactic acid population was detected in the epiphytic flora of pure sweet sorghum, and this situation was positively reflected in the number of lactic acid bacteria in the epiphytic flora of intercropping systems. The lactic acid bacteria of the intercropping systems were higher than those of the pure mung bean systems. Plants host many different microorganisms in their epiphytic flora and these microorganisms directly affects silage quality (Kung and Shaver, 2001). Wang et al. (2019) reported that lactic acid bacteria were predominant in alfalfa+sweet corn mixtures and pure sweet corn compared to pure alfalfa. Wang et al. (2017) found that even in maize harvest residues, the number of natural lactic acid bacteria was twice as high as in alfalfa and common vetch legume species. Similarly, the results regarding lactic acid bacteria counts obtained from this study were similar to the information highlighted above.

While the effects of years, Y×SP interaction, mixtures and SP×M interaction on plant enterobacteria numbers were significant, the effect of sowing patterns was insignificant (Table 1). The plant enthorobacteria numbers were determined as 4.93 log_{10}cfu g^{-1} DM in 2019, and 5.27 log_{10}cfu g^{-1} DM in 2020. Plant enterobacteria numbers varied between 4.87 log_{10}cfu g^{-1} DM and 5.39 log_{10}cfu g^{-1} DM in the interactions of years and sowing patterns (Figure 5). The highest number of enterobacteria was detected in narrow row in 2020 while the lowest was determined in the same sowing pattern in 2019 (Figure 5). The number of plant enterobacteria in the mixtures varied between 4.87 log_{10}cfu g^{-1} DM and 5.48 log_{10}cfu g^{-1} DM (Table 1). The highest number of plant enterobacteria was determined in the SS14 treatment. The lowest plant enterobacteria count was obtained from MB14 and MB21 treatments.

Table 1. Microbial changes in plant epiphytic flora according to trial years and treatments (sowing patterns and mixtures)

Treatments	Microbial Populations		
Years (Y)	Lactic acid bacteria	Enterobacteria	Yeast and Mold
2019	3.04±0.07 ^{a}	4.93±0.05 ^{b}	4.66±0.04 ^{b}
2020	2.96±0.07 ^{b}	5.27±0.05 ^{a}	4.79±0.04 ^{a}
P values (Y)	0.0046**	0.0120*	0.0007***
	Sowing patterns (SP)		
Conventional row	3.30±0.07 ^{a}	5.11±0.05	4.48±0.06 ^{b}
Narrow row	2.88±0.07 ^{b}	5.13±0.08	4.86±0.05 ^{a}
Twin row	2.84±0.09 ^{b}	5.05±0.07	4.84±0.04 ^{a}
P values (SP)	< 0.0001***	0.2340^{ns}	< 0.0001***
P values (Y×SP)	0.6556^{ns}	0.0261*	0.4392^{ns}
	Mixtures (M)		
MB14	2.37±0.06 ^{e}	4.87±0.08 ^{e}	4.28±0.04 ^{f}
MB21	2.03±0.08 ^{f}	4.87±0.10 ^{e}	4.42±0.08 ^{ef}
MB28	2.43±0.09 ^{e}	5.32±0.13 ^{ab}	4.78±0.07 ^{cd}
SS14	3.53±0.16 ^{a}	5.48±0.06 ^{a}	5.09±0.06 ^{a}
(R1:1)MB14+SS14	3.42±0.08 ^{abc}	5.16±0.07 ^{bcd}	5.05±0.08 ^{ab}
(R1:1)MB21+SS14	3.01±0.09 ^{d}	5.13±0.09 $^{b-e}$	4.75±0.09 ^{cd}
(R1:1)MB28+SS14	3.47±0.07 ^{ab}	5.08±0.08 $^{b-e}$	4.84±0.10 ^{bc}
(R1:2)MB14+SS14	3.21±0.06 ^{bcd}	4.98±0.13 ^{cde}	4.82±0.09 ^{bcd}
(R1:2)MB21+SS14	3.42±0.13 ^{abc}	4.90±0.22 ^{de}	4.58±0.12 ^{de}
(R1:2)MB28+SS14	3.16±0.10 ^{cd}	5.19±0.08 ^{abc}	4.65±0.05 ^{cde}
P values (M)	< 0.0001***	< 0.0001***	< 0.0001***
P values (Y×M)	0.9272^{ns}	0.7118^{ns}	0.2937^{ns}
P values (SP×M)	< 0.0001***	< 0.0001***	< 0.0001***
p values (Y×SP×M)	0.9822^{ns}	0.8093^{ns}	0.7460^{ns}
CV	7.77	4.00	4.16

^{a,b}Mean values with different superscripts in the same column indicate a significant difference (P < 0.05).

According to the SP×M interaction, enterobacteria numbers varied between 4.43 log_{10}cfu g^{-1} DM and 6.22 log_{10}cfu g^{-1} DM (Figure 6). The highest number of plant enterobacteria was determined in MB28 system of narrow row cultivation. The lowest plant enterobacteria count was obtained from MB28 application in twin row

cultivation. The plant enterobacteria numbers obtained from the present study were generally close to each other. Plant enterobacteria counts in pure sweet sorghum plots were higher than other treatments. Fresh plants contain many different microorganisms in their epiphytic (natural) flora, which can lead to the formation of a wide variety of end products in the silo (Kung and Shaver, 2001). Enterobacteria can sometimes cause ethanol production during the first 48 hours of fermentation in the silo, which is an undesirable feature in this case (Kung et al., 2018). It has been reported that the number of enterobacteria in the plant varies according to the growing conditions (Kung et al., 2018). This study showed that the enterobacteria count in sweet sorghum epiphytic flora was higher than in epiphytic flora of mung bean.

The effects of years, sowing patterns, mixtures and SP×M interactions on yeast and mold populations in plant epiphytic flora were significant ($P < 0.001$). While the number of yeast and molds was 4.66 log_{10}cfu g^{-1} DM in 2019, it became 4.79 log_{10}cfu g^{-1} DM in 2020 (Table 1). Among the sowing patterns, plant yeast and mold numbers were determined between 4.48 log_{10}cfu g^{-1} DM and 4.86 log_{10}cfu g^{-1} DM. The highest plant yeast and mold counts were obtained from narrow row and the lowest value was in conventional row. In addition, there was no statistical difference between narrow row and twin row planting treatments (Table 1). The number of plant yeasts and molds in the mixtures varied between 4.28 log_{10}cfu g^{-1} DM and 5.09 log_{10}cfu g^{-1} DM. The highest plant yeast and mold counts were obtained from the SS14 application. The lowest number of plant yeast and mold was determined in MB14 treatment (Table 1). Depending on the interaction (SP×M), the number of plant yeasts and molds varied between 3.89 log_{10}cfu g^{-1} DM and 5.20 log_{10}cfu g^{-1} DM. The highest plant yeast and mold numbers were obtained from (R1:1) MB14+SS14 treatment in double row. The lowest plant yeast and mold numbers were found in MB14 application in conventional row cultivation (Figure 7).

Although the plant yeast and mold numbers obtained from this study were generally close to each other but the statistical differences occurred within the applications. Yeast and mold density in plant epiphytic flora can sometimes result in high ethanol content in the silo, which can limit the aerobic stability of silages (Kung et al., 2018). Therefore, high yeast and mold populations in the natural flora of the plant are an undesirable feature. Wang et al. (2017) determined that the number of natural plant yeasts in corn harvest residues, which is a forage crop, was higher than in the legume type, similar to current study.

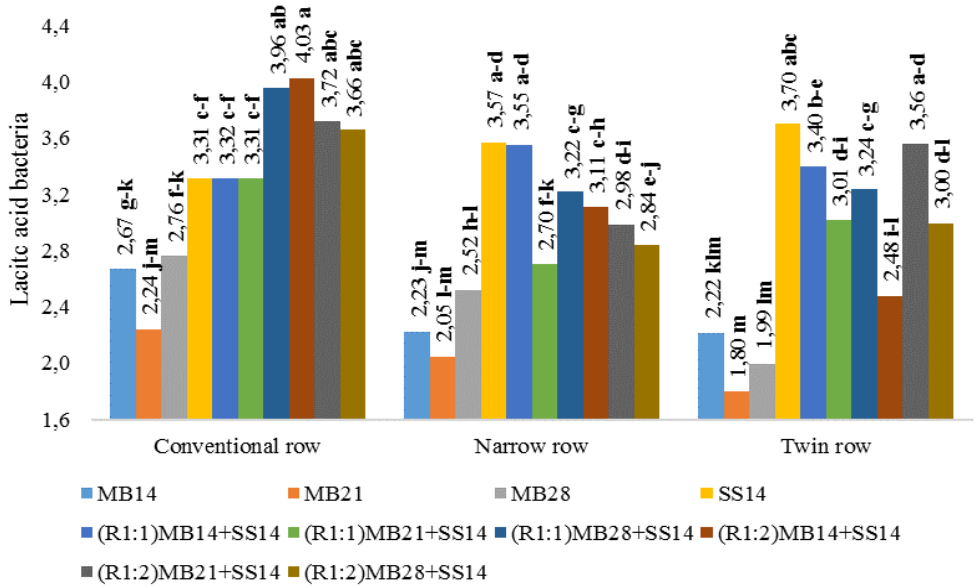

Figure 4. Lactic acid bacteria changes according to sowing pattern × mixture interactions.

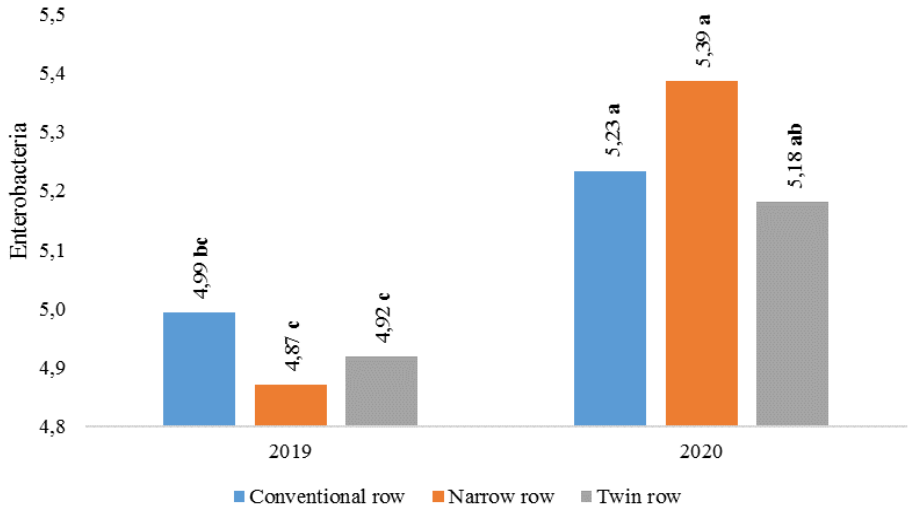

Figure 5. Entrerobacteria changes according to year × sowing pattern interactions.

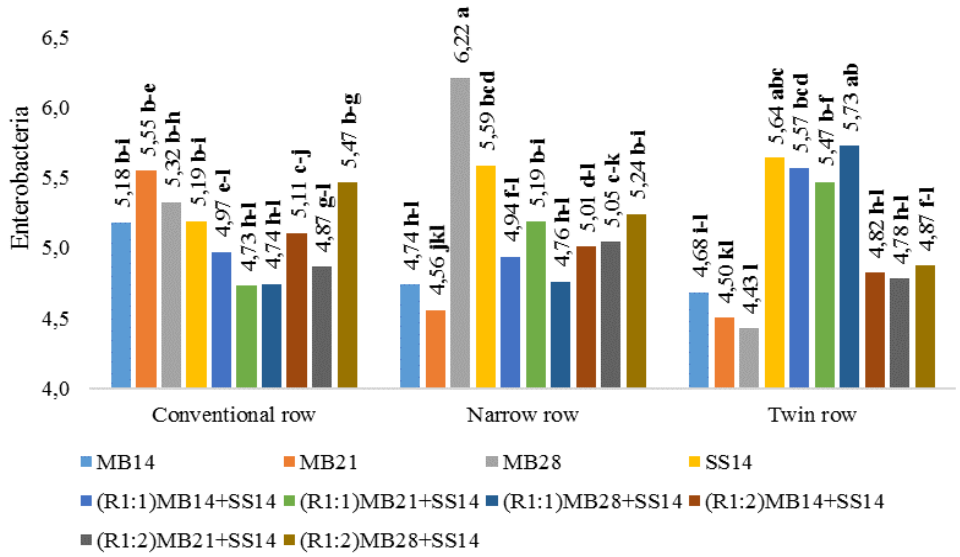

Figure 6. Entrerobacteria changes according to sowing pattern × mixture interactions.

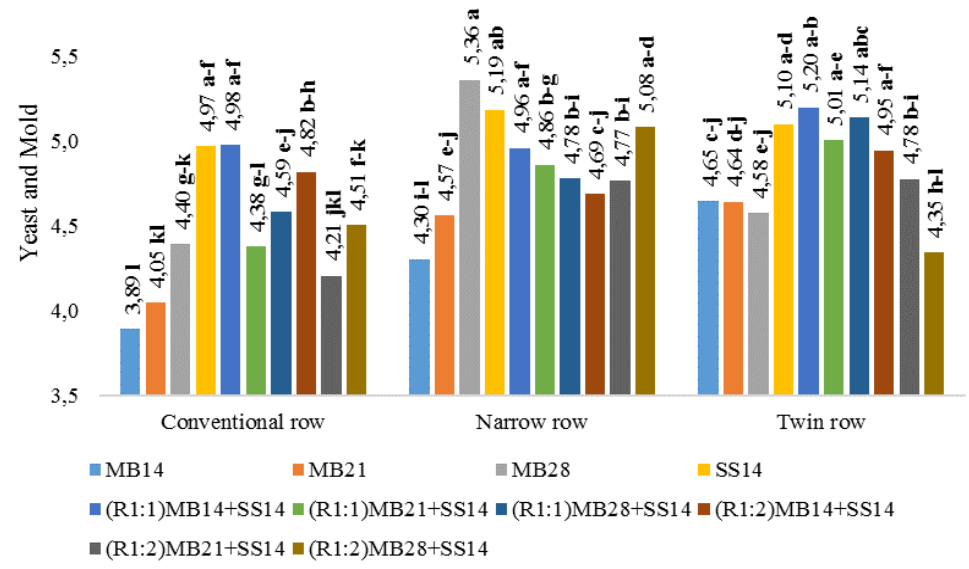

Figure 7. Yeast and mold changes according to sowing pattern × mixture interactions.

4. Conclusion

In this study, the change of microorganisms (lactic acid bacteria, enterobacteria, and yeast and molds) were investigated in mung bean and sweet sorghum grown with different sowing patterns and intercropping systems. The population of lactic acid bacteria, which has a positive effect on silage quality, was found to be higher in intercropping systems of conventional row method than others. On the other hand, enterobacteria, yeast and mold numbers, which are in the group of undesirable microorganisms for silage quality, were found to be lower in conventional row cultivation than others. Results from this study showed that intercropping systems of sweet sorghum and mung bean in conventional row method improved the population of lactic acid bacteria which is beneficial for silage quality.

Author Contributions

İ.E. and Ş.Y. wrote the manuscript and conceived the perspective, read, and approved the final manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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References

Ertekin I, Atis I, Aygun YZ, Yilmaz S, Kizilsimsek M. 2022. Effects of different nitrogen doses and cultivars on fermentation quality and nutritive value of Italian ryegrass (Lolium multiflorum Lam.) silages. Anim Biosci, 35(1): 39-46.

Ertekin İ, Kızılşimşek M. 2020. Effects of lactic acid bacteria inoculation in pre-harvesting period on fermentation and feed quality properties of alfalfa silage. Asian-Australas J Anim Sci, 33(2): 245-253.

Genç S, Soysal Mİ. 2018. Parametric and Nonparametric Post Hoc Tests. BSJ Eng Sci, 1(1): 18-27.

Kızılşimşek M, Erol A, Ertekin İ, Dönmez R, Katrancı B. 2016. Silaj mikro florasının birbirleri ile ilişkileri, silaj fermentasyonu ve kalitesi üzerine etkileri. KSÜ Doğa Bil Derg, 19(2): 136-140.

Kizilsimsek M, Ozturk C, Yanar K, Ertekin I, Ozkan CO, Kamalak A. 2017. Associative effects of ensiling soybean and corn plant as mixtures on the nutritive value, fermentation and methane emission. Fresenius Environ Bull, 26: 5754-5760.

Kung L-Jr. 2010. Aerobic stability of silage. 2010 California Alfalfa & Forage Symposium and Corn/Cereal Silage Conference, Visalia, CA, December 1–2, 2010, Davis, CA, US, UC Davis, pp. 1–14.

Kung L-Jr, Shaver RD. 2001. Interpretation and use of silage fermentation analysis reports. Focus on Forage, 3(13): 5p.

Kung L-Jr, Shaver RD, Grant RJ, Schmidt RJ. 2018. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. J Dairy Sci, 101: 4020-4033.

Santos MC, Lock AL, Mechor GD, Kung L-Jr. 2015. Effects of spoilage yeast from silage on in vitro ruminal fermentation. J Dairy Sci, 98: 2603-2610.

Wang M, Wang L, Yu Z. 2019. Fermentation dynamics and bacterial diversity of mixed lucerne and sweet corn stalk silage ensiled at six ratios. Grassl Sci, 74: 264-273.

Wang S, Yuan X, Dang Z, Li J, Shao T. 2017. Effect of ensiling corn stover with legume herbages in different proportions on fermentation characteristics, nutritive quality and in vitro digestibility on the Tibetan Plateau. Grassl Sci, 63: 236-244.

Yan Y, Li X, Guan H, Huang L, Ma X, Peng Y, Li Z, Nie G, Zhou J, Yang W, Cai Y, Zhang X. 2019. Microbial community and fermentation characteristics of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. Bioresour Technol, 279: 166-173.