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Biotechnological Impacts of Different Fungal and Bacterial Strains Treatment on Nutritive Value of Some Straws

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ABSTRACT

The current work was carried out to investigate the biotechnological impact of different strains of fungi (*Pleurotus Ostreatus*, Trichoderma viride and Trichoderma reesei) and bacterial treatment by LAB (Lactobacillus Plantarum with or without addition of molasses) on the nutritive value of agricultural residues for production of good quality straw silage. Four types of straws namely rice straw (RS), bean straw (BS), mixture of rice and bean straw (RBS) and wheat straw (WS) were subjected to biological treatment under solid state fermentation) with each Microorganism. The experimental groups include, control group of each substrate which did not inoculated with any microorganism (CON) , and five treatments groups , T1 (straws treated by Lactobacillus plantarum with molasses), T2 (straws treated with lactobacillus plantarum without molasses), T3 (straws treated by Trichoderma viride),T4 (Straws treated by pleurotus ostreatus) and T5 (Straw treated by Trichoderma reesei). Each treatment was carried out for four types of straws. The results of proximate analysis showed increased crude protein, ether extract and ash contents in all treatment compared to the un treated straws .The biotechnological treatment also led to reduction in dry matter, organic matter, crude fiber and fiber fractions contents in all treatments relative to the control group. So it is recommended to treat agricultural residues by cellulosic fungi and bacteria especially *Lactobacillus plantarum* for improving its nutritive value for animal feeding. Further investigations are required in vivo to study the effect of feeding of the produced biomass on the performance of ruminants.

1. INTRODUCTION

Nowadays, prices of concentrate as a feedstuffs used in animal feeding in Egypt increased dramatically, moreover the big feed gap between the requirements and the available sources motivate the nutritionists to look for non-conventional sources where there is no competition with humans. Agricultural by-products such as rice, bean and wheat straw are available all over the year but are not efficiently used (Abd El fattah, 2009). Due to there is no simple technique allow the utilization of these wastes, millions tons of carbohydrate remain unused as celluloltic wastes (Zaza, 2005). The relatively high content of lignin and cellulose in these residues is responsible for the limited digestibility, so many digestibility experiments and feeding trials (Khattab et al., 2011; Kholif et al., 2015) have been conducted to determine the nutritional values of crop residues and straws. They concluded that without treatment or nutrient supplementation, feeding of such residues can just, meet maintenance energy requirements. Many efforts have been attempts to increase digestibility of these lignocellulosic materials by physical, biological and chemical treatments (Eun et al., 2006; Dey et al., 2014; li et al., 2020). However, the studies indicated that the biological treatments are more effective in improvement the nutrient digestibility (Kabirifard et al., 2007; Khattab et al., 2009; Abdel-Aziz et al., 2014). Biotechnological approaches become essential to degrade lingo-cellulosics into lignin, cellulose and hemicellulose and increase crude protein content. The use of suitable microorganism (Lactobacillus plantarum, Trichoderma and pleurotus ostreatus) have been employed (Abdel-Aziz et al., 2014; El- Bordeny et al., 2015; Adebayo and Carrera,

2015, Zhao et al .2019). Fungi have the ability to utilize starch of the substrate to produce single cell protein (Pandey, 2003).Ensiling of crop residues with microorganisms such as lactic acid producing bacteria (LAB) and cellulolytic bacteria, resulted in improvement the nutritional quality of these residues as ruminant feed (Villas Boas et al., 2003).Moreover the LAB-treated silage had higher crude protein and organic matter, but lower water-soluble carbohydrates than did non treated silages. This method of straw treatment is believed to be safer than using of chemicals. Degradation of agricultural residues by solid state fermentation SSF (Microorganisms selected for SSF should have the ability to produce sufficient amount of appropriate enzymes that are able to degrade the cellulose and hemicelluloses in the substrate) has been considered (Zayed, 2018). By this method, lignin is preferentially decreased to zero percentage (Moyson & Verachtert 1991). So the objective of this study was to improve the nutritional values of some agricultural residues such as rice straw, bean straw and wheat straw by its biological treatment using celluletic fungi (Trichoderma viride, Trichoderma reesei and Pleurotus Ostreatus) and bacterial fermentation of the agricultural residues for silage production using lactic acid bacteria (LAB).

2. MATERIALS AND METHODS

The present study was carried out at the department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine; University of Sadat city. The microorganisms applied in our study are three fungal strains, two strain of *Trichoderma spp* (*Trichoderma viride and Trichoderma reesei*) and (*pleurotus ostreatus)* and one strain of lactic acid bacteria *(lactobacillus plantarum*). Fungal strains were obtained from Microbiology Research Center (MIRCEN), Faculty of Agriculture Ain Shams University**.** Strain of *Lactobacillus plantarum* obtained from Genetic Engineering and Biotechnology Institute, University of Sadat City, Department of Environmental Biotechnology. The agricultural substrate was obtained from the farm of University of Sadat City, Minoufia,Egypt.

2.1. Expremental design:

Four types of substrate; Rice straw (RS), Bean straw (BS), Wheat straw (WS), and Mixture of Rice & Bean straw (RBS) were included in this study. Straw was prepared and cleaned from any debris and dust then exposed to three types of fungi and one type of lactic acid bacteria. The experimental groups include, control group without treatment (CON), Straws treated by *Lactobacillus plantarum* with molasses (T1), Straws treated with *lactobacillus plantarum* without molasses (T2) , straw treated by *Trichoderma viride* (T3), Straw treated by *pleurotus ostreatus* (T4) and Straw treated by *Trichoderma reesei* (T5). Each treatment was carried out on the four types of Straws.

2.2. Fungal treatment of straws:

2.2.1. Preparation of straws:

Straw was chopped into small pieces (2-3 cm) and dried in the oven (55C) for 24hrs. Treatment was carried out in 500 ml jars (priviously washed, dried for 10 min. at 100°C). Twenty-five gram (25) g of the dried straw were weighed separately into jars and distilled water added to obtain moisture content of about 85%. The jars were immediately covered with aluminum foil and sterilized in the autoclave at 121°C for 15 min. Each treatment had five replicates (El-Ashry et al., 2002).

2.2.2 Preparing of fungi

Three types of fungi were used for treatment of straw in the present study namely *Trichoderma viride* (EMCC 107), *Trichoderma reesei* (EMCC 212) and *pleurotus ostreatus* (EMCC 603), were activated on specific medium (Potato Dextrose Agar) for 7-10 day at 25ºC (El-Ashry et al., 2002)

2.2.3. Inoculation of substrate with microorganism (solid state fermentation):

Solid-state fermentation of different straws was carried out by inoculation of each jar with1 g fresh mycelia weight from the fungal inoculants; however, the control group jars (CON) were not inoculated with any microorganisms. The Jars were incubated in an incubator in which temperature was adjusted to 25-30°C and 100% relative humidity (RH) for 4 weeks. At the end of the experiment samples were dried in oven (60°C) in order to stop fungi growth until a constant weight was obtained then stored in a refrigerator at 4 °C for chemical determination. **(**Wuanor and Ayoade, 2017).

2.3. Bacterial treatment of straw for silage production:

2. 3.1. Preparation of substrate:

Straws were cut into 2–3 cm pieces and moistened to 70–80% moisture content by spraying with water. straw pieces were compacted by hand as much as possible into 1 L jars, which were sealed with a lid and the joins filled with paraffin to prevent entry of air then sterilized in the autoclave at 121°C for 15 min. Each treatment was done in triplicates (Alves et al., 2011).

2.3.2. Preparation of bacterial inoculums (liquid submerged fermentation

The lactobacilli (de Man, Rogosa, Sharpe MRS) broth were inoculated with strain incubated overnight and the inoculum volume of LAB was 1 ml of

suspension per kilogram of FM (fresh material). The numbers of inoculated LAB 1.0 × 10^6 colony-forming units per (CFU) g of FM. The colonies of bacterial mixtures were counted by the dilution plate method. MRS broth (1L) consisted of 10 g peptone, 10 g beef extract, 5 g yeast extract, 2 g K2HPO4•3H2O, 2 g C6H14N2O7, 5 g NaAc, 20 g glucose, 1 mL Tween 80, 0.58 g MgSO4•7H2O and 0.25 g MnSO4•4H2O, pH = 6.2-6.4. Microbial inoculants were coated on MRS-S agar plates before inoculation to confirm their viability, and appropriate amounts of the inoculants were used to achieve the desired application rate (Pandey, 2003, Akinyele et al., 2012)

2.3.3. Silage preparation:

After preparation of bacterial inoculums, each type of straw was inoculated with two bacterial broth .first one (T1) was *lactobacillus plantarum* plus molasses (5% microbe juice and 5% molasses were mixed with 1 kg of straw based on dry matter) and the second treatment (T2) was *lactobacillus plantarum* only. Each jar filled with sterilized chopped straws and inoculated with bacterial broth till obtain60-70% moisture content .The jars were closed tightly with no air entry and stored at temperature 25-30°C for 30 day with repeated mixing, (Zhao et al .2019). Each treatment was done in five replicates.

2.4. Measurements of proximate chemical composition

DM was determined by drying milled samples to constant weight at 105°C overnight and ash was determined by igniting in muffle furnace at 550°C for 8 h (942.05. AOAC. 2002). OM was calculated by subtracting Ash from DM. EE was determined by soxhlet extraction method soxhlet extraction method (EE, method ID 920.39; AOAC 2002). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were measured by Fiber-Tec system (Van soest et al., 1991**)** Hemicellulose was calculated as the difference between NDF and ADF While, cellulose was calculated as the difference between ADF and ADL. Nitrogen (N) content was measured by the Kjeldahl method and crude protein (CP) was calculated as per N × 6.25 (CP, method ID 954.01; AOAC 2002). All the data was recorded on dry matter basis.

2.5. Statistical analysis:

The data obtained from these studies were subjected to analysis of variance and test (ANOVA) and test of significance was carried out by Duncan's multiple range tests using Statistical Analysis System (SAS.2004) package.

3. RESULTS

The results of this study reveal, significant decrease (P<0.05) in DM (Table 1) and OM (Table 2) contents of all types of the straws in all treatment groups relative to control. The highest reduction was recorded in bacterial treated group T1 (*Lactobacillus plantarum* with molasses) followed by T3 (*Trichoderma viride*). Furthermore, there was significant increase in ash content (Table 3) and decrease in CF (Table 5) contents of the straw in all groups compared to control one. The highest ash percentage and lowest CF % were estimated in T1 (*Lactobacillus plantarum* with molasses) followed by T5 (*Trichoderma reesei*).in addition, data in Table (4) represents, significant increase (P<0.05) in protein content of all treated straws in all groups compared to control group. The highest protein content was shown in T1 followed by T4 *pleurotus ostreatus*) and then T2 *(lactobacillus plantarum* without molasses). Moreover, the ether extract (EE) in all treated groups showed significant increase in all types of substrate compared to control (Table 6). The highest contents were present in T4 followed by T5 then T3. The results in Table (7), represents significant decrease in NDF percentage in all treated group relative to control. The greater reduction is reported in T1 followed by T5. significant decrease in ADF was detected in all treatment groups (Table 8), however the lowest content of ADF was presented in T5. Additionally, the data revealed significant reduction (P<0.05) in the content of hemicelluloses (Ttable 9), ADL (table 10) and cellulose (Table 11) in all groups when compared with the control one. The least amount in hemicelluloses and ADL content was achieved in T2 and T1 respectively while the lowest cellulose content was recorded in T5.

Table 1. Effect of Solid State and Submerged Fermentation on Dry Matter (DM) % of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	87.12±0.6^{a}	69.53±1.2^{d}	82.30±0.91b	79.20±0.9^{c}	84.73±0.99^{b}	82.99±0.62^{b}
BS	86.60±1.39^{a}	65.92±0.55^{d}	81.53±1.15^{bc}	79.05±1.20^{c}	84.71±0.87^{ab}	81.74±1.09^{b}
RBS	86.84±0.82^{a}	66.94±0.98^{d}	82.79±0.85^{b}	75.63±1.01^{c}	83.79±0.94^{b}	83.78±1.21^{b}
WS	85.42±1.14^{a}	65.08±0.81^{c}	84.27±0.84^{ab}	82.51±0.78^{b}	84.62±0.73^{ab}	84.62±0.49^{ab}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different (p < .05). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess , T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus*, T5: Straws treated by *Trichoderma Reesi*

Table 2. Effect of Solid State and Submerged Fermentation on organic Matter (OM) %of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	72.32±1.08^{a}	39.12±3.60^{d}	66.29±0.68^{bc}	62.86±0.31^{c}	68.87±0.75^{ab}	64.57±0.68^{bc}
BS	77.25±1.72^{a}	39.71±5.05^{d}	70.11±0.86^{ab}	65.08±1.56^{c}	73.34±0.72^{ab}	66.98±0.40^{bc}
RBS	77.97±0.77^{a}	42.27±0.85^{e}	72.56±0.80^{b}	62.46±1.50^{d}	73.28±1.45^{b}	67.30±1.26^{c}
WS	72.64±1.22^{a}	38.90±1.00^{c}	70.10±1.77^{ab}	67.11±1.40^{b}	70.49±0.61^{ab}	67.97±1.067^{b}

Mean± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

Table 3. Effect of Solid State and Submerged Fermentation on ash content (%) of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	14.79±0.67^{b}	30.41±3.76^{a}	16.01±0.69^{b}	16.34±1.48^{b}	15.59± 0.43^{b}	18.41±0.54^{b}
BS	9.34± 0.36^{d}	26.21±0.64^{a}	11.41± 0.34^{c}	13.25± 0.37^{b}	11.37± 0.52^{c}	14.49±0.37^{bc}
RBS	8.87±0.34^{d}	24.97±0.21^{a}	10.23±0.28^{c}	12.99±0.90^{d}	10.50±0.58^{d}	16.15± 0.33^{b}
WS	12.78±0.24^{d}	26.18±0.59^{a}	14.17±0.96^{cd}	15.40±0.36^{bc}	14.13±0.18^{cd}	16.83±0.62^{b}

Mea Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

Table 4. Effect of Solid State and Submerged Fermentation on crude protein (CP) % content of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	1.95± 0.11^{e}	20.56±0.25^{a}	7.81± 0.50^{c}	5.83±0.76^{d}	9.81±0.51^{b}	5.46±0.37^{d}
BS	6.71± 0.30^{d}	26.74±0.25^{a}	11.75±0.75^{b}	9.41±0.69^{c}	11.60±0.36^{b}	8.80±0.96^{c}
RBS	4.99±0.74^{d}	24.61±0.41^{a}	10.62±0.52^{b}	8.46±0.76^{c}	7.68±0.26^{c}	7.52±0.56^{c}
WS	4.36±0.45^{c}	24.15±1.06^{a}	8.00±0.52^{b}	6.23±0.94^{bc}	6.78±0.46^{bc}	6.46±1.17^{bc}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*.

Table 5. Effect of Solid State and Submerged Fermentation on crude fiber (CF)% content of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	48.37±1.40^{a}	9.20±0.86^{e}	35.91±1.79^{c}	30.75±3.78^{d}	42.41±0.60^{b}	26.68±0.34^{d}
BS	52.42±1.24^{a}	15.98±1.88^{e}	42.71±0.99^{bc}	40.86±0.56^{c}	45.26±0.67^{b}	37.38±0.61^{d}
RBS	47.69±1.17^{a}	10.08±1.40^{d}	38.85±0.34^{bc}	36.90±0.86^{c}	39.94±0.25^{b}	36.76±0.67^{c}
WS	45.88±0.23^{a}	9.93±1.02^{d}	34.49±0.41^{bc}	40.23±1.01^{c}	40.28±1.03^{b}	33.91±0.64^{c}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

Table 6. Effect of Solid State and Submerged Fermentation on ether extract (EE) % of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	1.66±0.15^{c}	3.62±0.34^{b}	2.11±0.106^{c}	3.65±0.27^{b}	4.70±0.18^{a}	3.90±0.072^{b}
BS	2.25±0.245^{d}	4.02±0.041^{b}	2.88±0.073^{cd}	3.51±0.43^{bc}	4.96±0.43^{a}	4.99±0.118^{a}
RBS	2.08±0.185^{c}	3.16±0.17^{b}	2.25±0.235^{c}	3.20±0.423^{b}	4.10±0.125^{a}	3.78±0.188^{ab}
WS	1.39±0.253^{c}	2.46±0.33^{b}	2.33±0.236^{b}	3.34±0.21^{a}	3.78±0.27^{a}	3.33±0.186^{a}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*.

Table 7. Effect of Solid State and Submerged Fermentation on Neutral detergent fiber % (NDF) of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	68.57±1.99^{a}	43.53±1.46^{c}	50.08±1.18^{b}	51.51±2.39^{b}	52.60±0.95^{b}	43.60±1.81^{c}
BS	71.01±1.15^{a}	44.61±1.07^{e}	50.65±0.29^{cd}	53.56±1.26^{bc}	54.90±1.26^{b}	49.43±0.88^{d}
RBS	62.46±0.419^{a}	40.98±1.057^{d}	45.31±0.565^{c}	53.80±1.74^{b}	52.45±0.804^{b}	45.73±0.337^{c}
WS	67.29±0.255^{a}	39.013±0.436^{d}	41.18±0.905^{d}	46.72±1.32^{c}	53.85±0.824^{b}	40.93±0.355^{d}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

Table 8. Effect of Solid State and Submerged Fermentation on acid detergent fiber % (ADF) of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	44.54±0.697^{a}	33.006±1.81^{cd}	36.076±1.45^{bc}	36.32±1.48^{bc}	37.32±0.492^{b}	31.60±0.803^{d}
BS	49.36±0.445^{a}	35.56±0.811^{c}	39.85±0.624^{b}	40.91±0.914^{b}	39.143±1.065^{b}	35.47±0.845^{c}
RBS	44.49±0.925^{a}	30.79±1.13^{d}	33.27±0.750^{cd}	39.16±1.61^{b}	39.00±1.078^{b}	34.426±0.795^{c}
WS	42.053±1.06^{a}	27.88±1.12^{b}	32.72±1.08^{b}	35.046±1.97^{b}	39.89±1.06^{ab}	31.48±0.674^{b}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess, T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

Table 9. Effect of Solid State and Submerged Fermentation on hemicelluloses % of different types of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	24.03±1.823^{a}	10.33±0.539^{c}	14.01±0.276^{b}	15.19±1.08^{b}	15.28±0.472^{b}	12.003±1.01^{bc}
BS	21.64±1.402^{a}	9.31±0.056^{d}	10.80±0.389^{d}	13.49±0.546^{c}	15.75±0.362^{b}	13.95±0.147^{bc}
RBS	17.97±1.152^{a}	10.38±0.475^{d}	12.036±0.184^{cd}	14.64±0.427^{b}	13.46±0.282^{bc}	11.30±0.472^{d}
WS	21.90±3.48^{a}	11.13±0.715^{bc}	8.46±0.737^{c}	11.67±0.737^{bc}	13.95±0.256^{b}	9.44±0.323^{bc}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

Table 10. Effect of Solid State and Submerged Fermentation on acid detergent lignin % (ADL) of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	10.69±0.347^{a}	3.90±0.402^{e}	8.15±0.358^{b}	6.48±0.468^{cd}	7.44±0.559^{bc}	5.18±0.437^{de}
BS	13.25±0.184^{a}	3.65±0.118^{d}	9.03±0.876^{bc}	8.136±0.284^{c}	9.56±0.348^{b}	8.11±0.108^{c}
RBS	9.99±0.216^{a}	2.74±0.192^{d}	8.20±0.562^{b}	6.89±0.238^{c}	6.56±0.415^{c}	7.15±0.102^{c}
WS	14.02±0.719^{a}	3.22±0.158^{e}	10.57±0.427^{b}	8.99±0.227^{c}	6.99±0.28^{d}	6.00±0.90^{d}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

Table 11. Effect of Solid State and Submerged Fermentation on celluloses % of straw

substrate	Treatments					
	CON	**T1**	**T2**	**T3**	**T4**	**T5**
RS	33.85±0.929^{a}	29.10±1.49^{b}	27.92±1.67^{b}	29.83±1.118^{b}	29.88±0.837^{b}	26.41±1.113^{b}
BS	36.11±0.481^{a}	31.91±0.802^{b}	30.82±0.756^{b}	31.62±0.362^{b}	29.57±1.282^{bc}	27.36±0.89^{c}
RBS	34.49±1.114^{a}	27.86±1.21^{b}	25.07±1.103^{b}	26.38±0.941^{b}	30.43±1.26^{b}	27.27±0.774^{b}
WS	32.90±1.20^{a}	24.66±1.035^{bc}	22.12±1.23^{c}	26.05±1.74^{b}	28.033±0.206^{b}	25.57±0.566^{bc}

Mean ± SE, a_ b_ c _d means within the same raw having different superscripts are significantly different ($p < .05$). (RS): Rice Straw (BS): Bean Straw (RBS): Mix of Rice and Bean Straw (WS): Wheat Straw. T1: Straws treated by *Lactobacillus Plantarum* with Molassess ,T2: Straws treated by *Lactobacillus Plantarum* without Molassess , T3: Straws treated by *Trichoderma Viridae* , T4: Straws treated by *Pleurotus Ostreatus,* T5: Straws treated by *Trichoderma Reesi*

4. DISCUSSION

The reduction in the content of dry matter in all used substrate in all treated groups (reach to 10% in RS treated with *Pleurotus Ostreatus* and 4.7 % with *Trichoderma viride* and *Trichoderma reesei*). is related to consumption of substrate carbohydrates in cell wall (Nasehi et al., 2017) as a result of fungal (EL-Tahan, 2003) or bacterial growth . this agree with El- Bordeny et al. (2015) who reported low DM content in rice straw treated biologically with *Trichoderma viride* and *Trichoderma reesei*. Moreover, the reduction of DM content in RS by (24.13%- 5.35%) in BS by (28.8% -5.8%), in RBS by (22.1%- 5.03%) and WS by (23% -1.3%) in bacterial treated groups, T1 and T2 respectively. Decreasing DM content of rice straw fermented with *lactobacillus plantarum* is recorded by Kim et al. (2017). However, Qin-hua et al. (2016) reported that treatment of rice straw by lactic acid bacteriaresulted in higher DM content of straw than un treated control one. The highest reduction in DM was found in *lactobacillus plantarum* group compared to fungal groups

The OM content decreased by (13.08%- 4.77%- 10.71%) in RS, (15.75%-5.06%-13.29%) in BS, (19.89%- 6.01 %- 13.68%) in RBS and (7.6%- 2.9%-6.4%) in WS in fungal treated groups, T3 ,T4 and T5 respectively. This reduction owing to breakdown in fibers of substrate while not affecting NFE (El- Ashry et al., 2002), this run in linear with the finding of previous researchers on RS (Jafari et al., 2007; Al-Samaraae and Alwaeli , 2016) treated with *Pleurotus ostreatus* , *Trichoderma* species respectively. Furthermore, biological bacterial treatment of straws (rice straw, bean straw, Mix of Rice and bean straw and wheat straw) by *Lactobacillus Plantarum*+ Molasses (T1) and *Lactobacillus Plantarum* without Molasses (T2) had Significantly ($P < 0.05$) lower OM content than untreated straw. The OM decreased by (45.9% - 8.33%) in RS, (48.5% - 7.14%) in BS, (44.86% - 6.93%) in RBS and (46.44%-3.45) in WS in T1 and T2 respectively. Li et al. (2016) concluded that, treatment corn steep liquor and air-dried rice with *L. plantarum*, *Lactobacillus casei*, and *Lactobacillus buchneri* resulted in decreasing in OM content. On other hand, when Ni et al. (2014) fermented wheat straw with *L. plantarum* for 30 days, he found no difference in OM content among treated and control groups. Confirming to our result concerning increase in ash content of by (10.48%- 5.4%-24.4%) in RS, (41.86%- 21.7%-55.13%) in BS , (46.44%- 18.37%- 82.07%) in RBS and (20.5%-10.56%-31.6%) in WS in T3, T4 and T5 respectively comparing to control. However Biological bacterial treatment of straws (rice straw, bean straw, Mix of Rice and bean straw and wheat straw) by *Lactobacillus plantarum*+ Molasses (T1) and *Lactobacillus plantarum* without Molasses (T2) had Significantly ($P < 0.05$) higher ash content than untreated straw. The ash increased by (105.6% -8.24%) in RS, (180.6%-22.16%) in BS, (181.5% - 15.33%) in RBS and (104.8% -10.87%) in WS in T1 and T2 respectively . Wichai and Songtong. (2017) reported an increase in ash content of Lactobacillus *plantarum* treated guinea grass by 4.6 % compared to control. Moreover, Abdel-Azim et al. (2011) concluded that treatment of rice straw and corn stalk with *Trichoderma Viride* increase their ash content. On contrary, Al-Samaraae and Alwaeli (2016) concluded that treatment of barley straw with (*Trichoderma harzianum*) did not have any effect on the amount of ash. Concluding that bacterial treatment of straws resulted in more improvement in ash content than fungal treated straws.

Biological fungal treatment of different types of straw significantly increase CP content by (198.9%- 403.07%- 180%) in RS , (40.23%- 72.87%- 31.14%) in BS ,(69.53%-53.9%- 50.7%) in RBS and (42.88%- 55.5%-48.16%) in WS in fungal treated groups, T3 ,T4 and T5 respectively than un treated straw. This could be explained by increase aerobic fermentation by fungus (Akinfemi, 2010),or presence of, extracellular enzymes, microorganisms and residual ingredients of media in the treated substrates (Siddhant and Singh 2009; Khattab et al., 2013), or proliferation of fungi during degradation (Akinfemi and Ogunwole, 2012) or attributed to the extracellular enzymes which secreted by the fungus that contain amorphous homo and hetero polysaccharides which associate with fungal protein (Abdel-Azim et al., 2011). This result is similar to that of Ramirez-Bribiesca et al. (2010) who found that P. *ostreatus* treatment for 15 days on corn straw increased crude protein (39.5%) and soluble protein (165%). Similar CP improvements of fungal treated straw were also found by Huyen et al (2019). Furthermore, Khattab et al. (2013) revealed that treatments rice straw with *Pleurotus ostreatus* increase its cp by (3.4 vs. 11.7%). Moreover, biological bacterial treatment of straw (rice straw, bean straw, Mix of Rice and bean straw and wheat straw) by *Lactobacillus plantarum*+ Molasses (T1) and *Lactobacillus plantarum* without Molasses(T2) had significantly($P<0.05$) higher CP content than untreated straw. The CP content increased by (925.6% -300.5%) in RS, (298.5% -75.11%) in BS, (393.18% -112.82%) in RBS and (453.89%- 83.48%) in WS in T1 and T2 respectively. Giving an indication that bacterial treatment of straw resulted in more improvement in straw CP than fungal

treatment. Kim et al. (2017) reported an increase in rice straw CP when treated with *Lactobacillus* owing that to lowering PH value which prevent protein degradation. (Li et al., 2014). However, Gado (1999) showed lowering in CP content in rice straw and baggas treated with *Trichoderma reesei*.

Data concerning CF,showed potentiality of *Lactobacillus plantarum* and *Trichoderma* species to decrease CF contents in different types of straws (80.97%-25.75%) in RS ,(69.51%- 18.52%) in BS ,(78.86%- 18.53%) in RBS and (78.35% -24.82%) in WS in bacterial groups, T1 and T2 respectively and (36.43%-12.32%- 44.84%) in RS ,(22.05%- 13.65%- 28.69%) in BS, (22.62%-16.25%- 22.9%) in RBS and (12.31% -12.20%-26.08%) in WS in fungal groups, T3 ,T4 and T5 respectively. One of the explanations for lowering CF from *lactobacillus plantarum* treatment is due to Enzymatic actions *e.g.* hemi-cellulase and cellulase in the original sbustrate degraded the cell wall during ensiling (Yahaya et al., 2004 ; de Oliveira et al. 2009). Or the fibrous component was hydrolyzed and many of the organic acids such as lactic acid and acetic acid were produced during ensilaging (Cao et al., 2010). In fungal treatment, CF reduction could be attributed to ability of fungal hypheeto penetratedeep into the cells of the straw and degrade CF(Akinfemi and OgunwOle, 2012) Similar trend was observed by other researchers(Li et al., 2016; El-Banna et al., 2010 ; Issaka el al., 2013) who reported a reduction in CF content of rice straw with *Lactobacillus plantarum*, *Trichoderma reesei* and *Pleurotus*-treated straw samples respecticely . This result proved that the bacterial treatment of straw give better reduction in CF than fungal treatment groups

Our data revealed significant improvement in EE content in different straws either fungal or bacterial treated. The higher EE content was recorded inT4 *(Pleurotus Ostreatus*) trated strawsfollowed by *Trichoderma* species where the The EE content increased by (119.87%- 183.13%-134.9%) in RS, (56%- 120.4%- 121.7%) in BS, (53.84% - 97.1%- 81.73%) in RBS and (140.28%- 171.9% -139.56%) in WS in T3, T4 and T5 respectively. These findings supported by Khattab et al. (2013), Akinfemi and OgunwOle, (2012) and Wuanor and Ayoade (2017) reports. Improvement of EE is probably associated to synthesis of fatty acids through growth of bacteria (Gado et al., 2007). Furthermore, increase EE content of straws by bacterial treatment is confirmed by other reports (Wichai and Songtong. 2017; Cao et al.2009; Gado et al., 2007). Conversely, Ni et al., (2014) indicated that the chemical composition and fermentation quality of wheat straw treated with LAB did not reveal any difference between treated wheat straw and control.

Regarding fiber fraction contents of different straws, the losses of NDF ADF and ADL contents in different types of straws due to fungal treatments denotes the ability of Fungi to degrade the cell walls as energy sources and so changed the percentage of insoluble to soluble carbohydrates in the straw (Khattab et al., 2013). And production of extracellular fungal enzymes (Akinfemi, 2010) similarly, El-Marakby (2003) noticed a great reduction in the content of NDF, ADF, cellulose and hemicellulose of wheat straw treated with white rot fungi. In consistent to this results, El-Banna et al. (2010) and Islamiyati et al.(2013) El- Bordeny et al. (2015) observed that treatment of sugar cane , corn stover and rice straw with *Trichoderma reesei* , *viride* and both of them respectively lowered straws content from NDF ADF, ADL, cellulose and hemicelluloses. Similar results were indicated by Vorlaphim et al (2018) Moreover, Akinfemi and OgunwOle. (2012) indicated that rice straw treated with *Pleurotus ostreatus* (POR), *Pleurotus pulmonarius* (PPR) and *Pleurotus tuber-regium* (PTR) significantly affect cellulose, NDF, ADF and ADL. in linear, Khattab et al. (2013) observeed that treatments of rice straw with *Pleurotus ostreatus* greatly reduced content of NDF (63.5 vs. 39.6%) , ADF (36.2 vs. 30.2%), ADL (9.4 vs. 4.3%), hemicellulose (27.2 vs. 9.4%), cellulose (25.9 vs. 26.9%). On contrary, Al-Samaraae and Alwaeli ,(2016) found no significant effect on the amount of cellulose, NDF and ADF of barley straw treated with *Trichoderma harzianum* .

Bacterial treatments of all substrates with *Lactobacillus plantarum*+ Molasses (T1)and *Lactobacillus plantarum* without Molasses(T2) greatly decrease ($p<.05$) their content of NDF, ADF, Cellulose, Hemicellulose and Lignin comparing to control and fungal treated groups as a result of degrading the crude fiber during fermentation process (Guan et al., 2002). In addition presence of Molasses, significantly decreased NDF and ADF, due to acid hydrolysis of cell walls carbohydrates as a result of decreasing silage pH by lactic acid fermentation (Yuan et al., 2016). In the same pattern Li et al. (2010) reported that inoculation of rice straw silage with *Lactobacillus plantarum* results in decrease its content of NDF and ADF. Furthermore, Li et al. (2016) found that fermentation of corn steep liquor and air-dried rice straw with a group of homo-fermentative and hetero-fermentative *Lactobacillus* which were *L. plantarum*, *Lactobacillus casei*, and *Lactobacillus buchneri* significantly reduce the

content of NDF and ADF to 54.82% and 34.12%, respectively.

Conclusions

It was concluded that Fungal and bacterial treatment of different type of Straws (rice straw, bean straw, mix of rice and bean straw and wheat straw) by Mushroom spp. (Pleurotus ostreatus) or Trichoderma spp. (Trichoderma viridae and Trichoderma reesi) and (Lactobacillus plantarum with or without molasses addition) improve the nutritive value of Straws. However the best and highest nutritional value for all types of substrate was found in the group treated with *Lactobacillus plantarum* with molasses.

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