Biotechnological Improvement of Nutritional Value of Rice straw using Fungi

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Abstract

Background

Elimination of most of Egyptian environmental pollution depends on prevention of burning of agricultural wastes specially rice straw. Microbial improvement of nutritive value for rice straw is not only aids in the prevention of Egyptian environmental pollution but also solve the problem of shortage in animal feeds ingredients. The current study was designed to investigate the microbial improvement of rice straw via solid state fermentation using five strains of fungi namely *Trichoderma viride*, *Trichoderma reesei*, *pleurotus ostreatus*, *Aspergillus oryzae* and *Aspergillus Fetidus*.

Results

This fermentation results in improving of nutritive value of rice straw by increasing its dry matter, protein, fat, ash and energy content with decreasing of fiber and organic matter content. These effects were variable according the type of fungi, which were very high for rice straw treated with *Trichoderma viride* and *Trichoderma reesei* followed by that treated by *pleurotus ostreatus* then those treated by *Aspergillus oryzae* and *Aspergillus Fetidus*.

Conclusion

It could be concluded that the microbial treatment is a good way to improve the nutritional value of rice straw

Trial registration

Project Nr.9/2017- Faculty of Veterinary Medicine- University of Sadat City.

<http://usc.edu.eg/ar/news/13741.aspx>

Background

In Egypt the annual agriculture by-products estimated to be around 30 million tons of dry material [44]. Approximately two thirds of the crop residues are burned or wasted, and hence lead to environmental pollution and consequently health hazards. Burning of agricultural wastes specially rice straw form 42% of Egyptian environmental pollution [38]. Utilization of such by-products can not only be used in favor of solving animal feed shortage problem but also as a method to control environmental pollution [69]. To increase the nutritive value of rice straw, many efforts have been employed. Several reports have been documented on the use of chemical, physical, mechanical and biological treatments [34], [58]. Biotechnological approaches as the use of suitable microorganism have been employed [59]. This approach is believed to be more safe and eco-friendly than using of chemicals. Recently, biological degradation of agricultural residues by solid state fermentation (SSF) using selected microorganisms should have the ability to produce sufficient amount of appropriate enzymes that are able to degrade the

cellulose and hemicelluloses in the substrate [23], [37]. By this method, lignin is preferentially decreased to zero percentage [39].

Fungal organisms have the ability to utilize starch of the substrate to produce single cell protein [47], [42]. However, the reduction of lignin and lignocellulosic complex depend on the strain of fungi and the suitable cultural conditions [63]. Among different fungal strains, treatments with *Asprigillus spp* increase crude protein of different substrates as rice bran, sugar cane and corn cob [64], [65]. Furthermore, several reports revealed the ability of certain species of fungi (white rot fungi *Pleurotus Sajor*) to enhance the nutrients in both stem and leaves of rice straw within 30 days incubation period [29]. Moreover, treatment of agricultural wastes with *Pleurotus* species resulted in increased crude protein, Ether extract and mineral content, while decreasing crude fiber and cell wall constituents [31]. The ability of White-rot fungi in degrading lignin returned to secreting enzymes collectively termed “ligninases” [35]. Furthermore, [7] found that rice straw treated with three different edible mushrooms: *Pleurotus ostreatus* (POR), *Pleurotus pulmonarius* (PPR) and *Pleurotus tuber-regium* (PTR) resulted in increase in crude protein and reduction of crude fiber fractions of the treated rice straw. Another strain of fungi known by its capacity in degrading indigestible fiber of rice straw by secreting lytic enzymes are *Trichoderma* which commonly found in all climatic zones. Cellulases, hemicellulases, and pectinases produced by *Trichoderma* fungi are used in the partial hydrolysis of plant cell walls in animal feeds, enhancing its digestibility and nutritive value [68], [53]. So the aim of the current study was to differentiate nutritionally among the effects of certain strains of fungi and their ability to enhance the quality of rice straw and improving its nutritional value for animal feeding.

Methods

Preparation of Substrate

The agricultural substrate (rice straw) was obtained from the farm of University of Sadat City, Minufia, Egypt, then chopped into small pieces (2-3 cm) and dried in the oven (55°C) for 24 hrs [60]. Treatment was carried out in 500 ml jars (washed and dried for 10 min. at 100°C). Twenty-five gram 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 then sterilized in autoclave at 121°C for 15 min. The experiment was designed to be five replicates for each treatment.

Preparation of Fungal Inoculum

Five types of fungi (Egypt Microbial Culture Collection EMCC) were used for treatment of rice straw in the present study namely; *Trichoderma viride* (EMCC 107; DSM63065, IMB12098), *Trichoderma reesei* (EMCC 531; ATCC28217,NRRL3653, QM9123) and *pleurotus ostreatus* (EMCC 603; ATCC56270,NRRL 3501), *Aspergillusoryzae* (EMCC 163; ATCC56747,DSM1863, IMI 283863, NRRL 3488) and *Aspergillus Fetidus* (EMCC 123; ATCC10254, Biourge739, a.citricus culture, cbs 126.48, DSM 734 IMI15954, 41871). They were obtained from Microbiology Research Center (MIRCEN) of the Faculty of Agriculture in the University

of Ain Shams, Egypt. Fungi were firstly activated and cultivated on specific medium (Potato Dextrose Agar) for 7-10 day at 25°C [17] as shown in Fig.1.

Experimental Layout

Six treatments were applied with five replicates including; rice straw without fungal treatment (Control), rice straw treated with *Trichoderma viride* (T1), rice straw treated with *Trichoderma reesei* (T2), rice straw treated with *Pleurotus ostreatus* (T3), rice straw treated with *Aspergillus oryzae* (T4) and rice straw treated with *Aspragillus Fetidus* (T5).

Fungal Inoculation (Solid State Fermentation)

Solid-state fermentation of rice straw was carried out by inoculation of each jar with 1g fresh mycelia weight from the fungal inoculants. The Jars were incubated in an incubator in which temperature was adjusted to 25-30°C and 100% relative humidity for 4 weeks. At the end of the experiment samples were dried in oven (60°C) for 24h in order to stop fungi growth then dried at 135°C for 2hrs until a constant weight was obtained then stored for proximate chemical analysis [67] as shown in Fig.2.

Proximate Chemical Composition

Dry matter (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 6- 8 h according to method 942.05 [11]. Organic matter (OM) was calculated by subtracting ash from dry matter. Ether extract (EE) was determined by soxhlet extraction according to method 920.39 [11]. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were measured by official method Ba 6a-05 using filter bag technique (AOCS 2009). Fiber-Tec Ankom system [12], [66]. 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 $N \times 6.25$ according to method 954.01 [11]. All data were recorded on dry matter basis.

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 package [57].

Results

Table (1) and (2) show the statistical results data of our work. The results indicate that the fungal treatment of rice straw using solid state fermentation increased its crude protein, fat, energy and ash content while decreasing its dry matter and fiber content in all groups compared with control group. On the other hand, its content for crude fiber reduced in group T1, T3, and T5 but not affected in groups T2 and T4 compared with control group. Also, its content for NDF and ADF decreased in groups T1, T2, and

T3 but not affected for biomass for groups T4 and T5, while organic matter also reduced in biomass for groups T1 and T3.

Table 1 Effect of fungal treatment on the nutritional value of rice straw

Items	Control	T1	T2	T3	T4	T5
DM%	91.34 ± 1.8 ^a	82.46 ± 0.91 ^c	82.46 ± 0.99 ^c	83.0 ± 0.62 ^c	85.0 ± 0.39 ^a	85.6 ± 0.32 ^b
Ash%	14.9 ± 0.5 ^c	16.02 ± 0.4 ^{bc}	15.60 ± 0.4 ^c	18.42 ± 0.5 ^a	17.40 ± 0.09 ^{ab}	17.60 ± 0.6 ^a
OM%	76.5 ± 2.02 ^a	66.29 ± 0.7 ^{bc}	68.87 ± 0.75 ^b	64.57 ± 0.68 ^c	77.60 ± 0.34 ^a	77.93 ± 0.30 ^a
CP%	1.95 ± 0.11 ^c	7.81 ± 0.50 ^a	9.81 ± 0.51 ^a	5.50 ± 0.37 ^b	5.83 ± 0.16 ^b	6.50 ± 0.50 ^b
EE%	1.67 ± 0.15 ^e	2.12 ± 0.10 ^d	3.90 ± 0.18 ^b	4.70 ± 0.07 ^a	2.57 ± 0.08 ^c	2.70 ± 0.11 ^c
CF%	48.38 ± 1.4 ^a	35.91 ± 1.7 ^b	42.41 ± 0.6 ^a	26.68 ± 0.3 ^c	37.19 ± 1.1 ^b	34.16 ± 1.1 ^c
NEL * cal/lb	0.37 ± 0.01 ^d	0.63 ± 0.02 ^b	0.69 ± 0.01 ^a	0.62 ± 0.01 ^b	0.41 ± 0.01 ^{cd}	0.42 ± 0.01 ^c
TDN* %	38.4 ± 0.7 ^d	61.4 ± 1.6 ^b	66.5 ± 0.90 ^a	60.0 ± 0.6 ^b	41.8 ± 1.6 ^{cd}	42.4 ± 0.5 ^c

*NEL (Mcal/lb) = 1.0876 – (0.0127 x ADF)

*TDN% = 4.898 + (89.796x NEL)

Table 2 Effect of Fungal Treatment on the Fibre Fractions of Rice Straw

Items	Control	T ₁	T ₂	T ₃	T ₄	T ₅
NDF%	70.1 ± 0.8 ^a	49.5 ± 0.4 ^c	51.53 ± 0.7 ^c	42.6 ± 1.2 ^d	68.8 ± 1.2 ^{ab}	66.71 ± 0.7 ^b
ADF%	56.3 ± 0.6 ^a	36.1 ± 1.4 ^c	37.33 ± 0.5 ^c	31.6 ± 0.8 ^d	53.3 ± 1.4 ^{ab}	52.80 ± 0.4 ^b
Hemicellulose%	24.0 ± 1.8 ^a	14.0 ± 0.3 ^{bc}	15.28 ± 0.5 ^b	12.0 ± 1.0 ^c	15.5 ± 1.0 ^b	13.91 ± 0.4 ^{bc}
ADL	10.7 ± 0.3 ^a	8.2 ± 0.4 ^b	7.45 ± 0.6 ^b	5.2 ± 0.4 ^c	10.1 ± 0.8 ^a	10.47 ± 0.3 ^a
Cellulose %	33.9 ± 0.9 ^a	27.9 ± 1.6 ^{ab}	29.9 ± 0.8 ^b	26.4 ± 1.1 ^c	41.9 ± 1.5 ^b	42.27 ± 0.2 ^{ab}

Discussion

The filamentous fungi especially *Aspergillus* and *Trichoderma* spp., are highly efficient producers of cellulases [49] and increase the total biomass [54]. Inoculation of substrate by *Aspergillus oryzae* accumulates monosaccharides for conversion into mycelial biomass during aerobic fermentation [27]. Chemical composition of untreated control straw and biological treated rice straws are presented in table 1. Biological treatments using fungal strains decrease dry matter (DM) content of rice straw significantly ($P < 0.05$). The highest value of reduction in DM content was recorded in T1 (*Trichoderma viridae*), T2 (*Trichoderma reesei*) and T3 (*pleurotus ostreatus*) respectively compared with other groups. These reductions in DM content are result of hydrolysis effect of fungi which decompose the solid substrates of straw, which could be correlated with consumption of carbohydrates in the cell wall [43]. In addition, this loss in DM content is related to the extent of fungal growth [40]. This result was in linear with the finding of [19] who reported that biodegradation of rice straw by *Trichoderma viride* and *Trichoderma resei* lead to reduction of DM content. Moreover, [30] reported that biological treatments of rice straw with *Pleurotus ostreatus* decreased content of DM (83.1% vs. 93.1%). Also, [41] revealed that paddy (rice) straw treated with *Trichoderma viride* had a lower content of dry matter due to difference in the degradation of structural carbohydrates. In addition, [15] found a decreased dry matter of rice straw treated with *Aspergillus Oryzae* and [62] stated lower effect because of reduced enzyme production for *A. Foetidus*. On other hand, [39] and [4] found that the dry matter content was increased by 28.21 % in wheat straw treated with *Pleurotus ostreatus* mycelium for 120 days.

As shown in table (1). There are significant increases ($P < .05$) in ash contents of rice straw of all treatments compared to control one. The highest ash content was recorded in T3 (treated by *P. ostreatus*), T4 (treated by *Aspergillus Oryzae*) and T5 (treated by *Aspergillus Foetidus*) followed by T2 (treated by *Trichoderma reesei*) and T1 (treated by *Trichoderma viridae*), which could be resulted from utilization of OM by enzymes secreted by fungi, these fungi are unable to obtain their requirements of carbons and energy requirements from lignin of rice straw, and therefore, require other substrates for carbons and

energy such as cellulose or hemicellulose for their growth and delignification, so increase ash content [26]. These findings agree with other previous researchers [19], [1]- [18] who reported an increase in ash content of rice straw treated with *Trichoderma viride* and *Trichoderma reesei*, than control one. Moreover, [51] reported that *P. ostreatus* treated corn straw for 15 days increased ash content by 188.32% than untreated one. In an article of [40] stated an increased inorganic matter as decreased organic matter by using *Aspergillus Oryzae* in feeding sheep. On contrary, [9] concluded that treatment of barley straw with fungi (*Trichoderma harzianum*) did not reveal any significant effect on ash content.

Results in table (1) revealed that fungal treatment of straw significantly decrease ($P < .05$) organic matter (OM) content of rice straw in T1, T2 and T3 compared to control one but increased with *Aspergillus* (T4 and T5).

The reduction of OM content may be attributed to utilization of soluble and structural carbohydrate by fungal cells as carbon source to produce energy and CO₂ for fungal biomass growth. this run in accordance with [19] and [1] for *trichoderma viride* and *trichoderma reesei* and with [30] and [26] for *Pleurotus ostreatus* which decreased content of OM of rice straw compared to untreated one. Reference [6] found the same result with SSF using *Aspergillus spp.* However, [10] found no effect on OM of barley straw treated with *Trichoderma harzianum* and did not reveal any significant effect on ash content.

Our results in table (1) revealed improvement of crude protein content from 3 to 5 times in all fungal treated groups compared to control one. Where the highest protein contents were for T2 (treated by (*trichoderma viride*) followed by T1 (treated by (*trichoderma reesei*). This improvement could be attributed to extracellular enzymes and residual media ingredients in the straw [60] and [30] or may be due to aerobic fermentation [7] and proliferation of fungi during degradation [8] or bioconversion of carbohydrates as a source of energy with nitrogen source in media, into single cell protein by the growing fungus during the fermentation process [25]. This finding agree with [5] who concluded that Crude protein increased significantly throughout the incubation period from 6.65% to 14.82% in rice straw treated by *Pleurotus pulmonarius*. Furthermore, [13] reported that wheat straw treated with *Pleurotus ostreatus* succeeded to increase protein content from 3.30% to 10.85. Moreover, [2] revealed that treated rice straw with *Trichoderma viride* increased CP from 3.9% to 9.45%. This result was supported by other researchers [1], [24]- [19] who found that treatment of rice straw by *Trichoderma viride* and *Trichoderma reesei* increase the protein content by 91.7% and 28.5% respectively. Also, [33] found an increased Nitrogen intake of sheep by increasing fermentable biomass using *Aspergillus spp.* On other hand, [21] and [10] found that treatment of rice and barley straw with *Trichoderma* have no effect on the amount of CP.

Data in table (1) show significant increase ($P < .05$) in ether extract (EE) content of rice straw in all treatments compared to control one being highest in T2, followed by T3, T4 and T5 while the least in T1. Reference [52] reported that the products of solid state fermentation gets in enriched fats, vitamins, soluble sugars and amino acids which can be utilized entirely as in animal feed. In the same trend, [53], [1], [30] and [8] concluded that treatment of rice straw with *Trichoderma Viride* and *Pleurotus ostreatus*, significantly increased ether extract of treated rice straw. In reference [67] rice straw treated with three

strains of edible mushroom, *Pleurotus tuber regium*, *Pleurotus pulmonarius* and *Pleurotus ostreatus* and he found that ether extract was significantly ($P = 0.01$) affected. In reference [70] was found an increased lipids production with SSF using *Aspergillus spp.* On the other side, [18], [21] and [32] reported reduction in EE of straw treated with fungi.

It is possible to accelerate decomposition of fibrous materials using cellulolytic/lignolytic microorganisms like *Trichoderma*, and *Aspergillus spp.* [56]. As presented in table (1), fungal treatments of straw reduce significantly ($P < 0.05$) crude fiber (CF) content than untreated one. This reduction due to that fungi have enzymatic potential to use lignocellulose component as sources of carbon and energy as indicated by [9] and its effective in degradation of CF because the hyphae of these fungi were capable of penetrating deep into the cells of the straw [8], so not only grow on the surface but also penetrated deep into the substrates. This observation is consistent with [30] and [8] who found that rice straw treated with *Pleurotus ostreatus* decrease amount of crude fiber from 32.89 % in control to 19.96 % in treated group. Moreover, [36] indicated that, CF of sugar cane bagasse was improved after being treated with fungi (*Trichoderma reesei*) and decreased from 41.3% in untreated sugar cane bagasse to 31.07 % in treated one, who supported by the finding of [18]. Reference [62] concluded more hydrolysis for lignocellulosic biomass of straw using *Aspergillus spp.* On other point of view, [5] and [10] reported no effect of *Pleurotus pulmonarius* or *Trichoderma harzianum* on cotton waste and barley straw respectively. The determination of feed value is currently made through chemical and biological analyses. However, chemical analyses cannot adequately measure the real value of animal feeds; The actual feed quality depends on an evaluation of the nutrients in the context of the animals being fed [28]. In the light of above information, we can conclude that the improvement in the nutritive value of rice straw by rising of its content of protein and fat with decomposition of lignocellulytic compounds lead to increasing of its energy value as shown in table (1), the energy value (calculated TDN and NEL) of rice straw [46] increased significantly ($P < 0.05$) in T1, T2, and T3, which followed by T4 and T5 compared with that of control untreated straw [30] and [51]. The efficiency of *Aspergillus* and *Trichoderma* in producing and secreting a broad range of cellulases and hemicellulases is industrially used to produce many pectinases [14], [48] and hemicellulases [50], [15]. This efficiency could be recognized by the reducing effect of these enzymes on fiber content of straw and its fraction. Results in table (2) indicated significant reduction ($p < .05$) in NDF, ADF, Cellulose, Hemi-cellulose and ADL contents of treated rice straw than un treated one being least in *pleurotus osteratus* treated group (T3) followed by *trichoderma* (T1 and T2) and *Aspergillus* T4 and T5). The lower contents of NDF, ADF and ADL contents of rice straw suggested the ability of Fungi to solubilize cell walls as carbon sources and thus changed the ratio of insoluble to soluble carbohydrates in the straw [30].

These findings were in agreement with that of [45] who found that treatment of wheat straw with *Pleurotus ostreatus* for 70 days decreases the lignin and cellulose content from 10.53 and 37.12% to 7.32 and 34.13 %, respectively. Furthermore, [20] noticed that white rot fungus *Agaricus bisporous* greatly decrease content of neutral detergent fiber (NDF-by 45.1%), acid detergent fiber (ADF- by 31.5%), cellulose (by 53.7%), and hemicellulose (by 96.3%) in wheat straw. The results were also in consistent with that of [19], [24] and [18] who found that sugar cane bagasse treated with brown rot fungi (*Trichoderma reesei* F-

418) decreases the content of NDF (73.1 vs. 59.9), acid detergent lignin (ADL, 20.2 vs. 25.8), ADF (51.6 vs. 45.8), cellulose (31.4 vs. 21.1) and hemicellulose by 27.3%. Moreover, [55] reported that fungal-treated rice straw reduced the NDF and ADF content from 79.54 to 74.02% for NDF and 63.69 to 59.74% for ADF, respectively. [62] found an increased hydrolysis of lignocellulosic biomass of straw using *Aspergillus spp.* On the other side, [10] found that treatment of barley straw with *Trichoderma harzianum* did not have any significant effect on the amount of cellulose, neutral detergent fiber, and acid detergent fiber.

Conclusions

It could be concluded that the microbial treatment is a good way to improve the nutritional value of rice straw for reducing the Egyptian environmental pollution, where the solid state fermentation of rice straw using fungi improved its nutritive value. Further studies are needed to use other microbes to increase its nutritional value.

Declarations

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Authors' contributions

OMA and AAA performed the experimental and laboratory work. MFS and AY worked on the analysis and interpretation of the data and contributed with valuable discussions. KMG and RA conceived the project, worked on the structure and wrote the paper. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

TrichodermaVirid

Trichoderma reesei

Pleurotus ostreatus

Aspergillus Oryzea

Aspergillus Foetidus

Figure 1

Fungal growth for different strains

Figure 2

Fungal biomass for rice straw