The Journal of Animal & Plant Sciences, 26(2): 2016, Page: 309-314
ISSN: 1018-7081

OPTIMIZATION OF SOLID STATE FERMENTATION CONDITIONS USING *ARACHNIOTUSSPECIES* FOR PRODUCTION OF FUNGAL TREATED WHEAT STRAW

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ABSTRACT

The study was carried out to determine the effects of optimum cultureconditions (substrate to water ratio, incubation period and salt concentrations, e.g. $MgSO_4.7H_2O$, $CaCl_2$, KH_2PO_4 and urea) to increase the nutritive value of wheat straw (WS) by solid-state fermentation (SSF) with *Arachniotus species.* The performance of SSF product was evaluated in terms of favorable change in the crude protein (CP) contents of WS. Wheat straw based inoculum (seed culture) of *Arachniotus sp.* was prepared. WS (5gm) in 250 mL conical flask was mixed with a basal medium containing selected salts to attain the desired levels of water and nutrient concentration. A significant (p<0.001) increased CP contents was observed at 1:2 substrate water ratio at 4th day of incubation period.Maximum fungal protein contents in treated wheat strawwere found by addition of 0.05%, 0.075%, 0.15% and 0.15% of$MgSO_4.7H_2O$, $CaCl_2$, KH_2PO_4 and urea respectively. Itwas thus concluded that enhanced protein contents were achieved by incubation of wheat straw at 1:2 substrate water ratio with our selected salts concentrations during 4 days of incubation at 28°C.Hence,our findings highly support the use of above salts at our optimized concentrations for bioprocessing of wheat straw with *Arachniotusspecies*.

Key words: Wheat straw, Salts concentrations, Fungi, Solid state fermentation, *Arachniotus.*

INTRODUCTION

Milk and meat production in Pakistan have shown an increasing trend over the last several years that is due to increase in total number of animals rather than per head production. Feed scarcity, inadequate and unbalanced nutrition and subsequent animal feeding with dry roughages are substantial constraints of low livestock production. Although efforts have been made to increase the overall production potential but there is anutmost need to properly utilize the available feed resources. For this purpose, nutrition management entails anefficient utilization of abundantly available crop residues (46%), that already gained attention as atreated feed for animal consumption in developing countries (Hasnain and Usmani, 2006). However, low nutrients digestibility, low protein and high fiber contents limit their utilization in ruminant feed (Abo-Eid *et al.,* 2007; Khan and Chaudhry, 2011).

The poor quality of these crop residues like wheat straw (WS) could be improved through novel methodologies involving biotechnological approaches. For successful application of these methods, an important step is the selection of the most appropriate microbesthat have the ability to grow on amoist substrate and in aerobic conditions through solid-state fermentation (SSF). This technology is used for degradation of cell wall components like cellulose, hemicellulose and lignin by fungal application, to acquire increased digestibility of low quality WS (Shahzad *et al.,* 2013).

Different kinds of fungi have been used in SSF to improve the nutritive value of industrial by-products or highly fibrous agricultural wastes (Chalamcherla *et al.,* 2009). Growth of fungal mycelium was previously found to enhance total protein contents in fermented feed (Fazaeli and Mirhadi, 2007). However, long incubation periods and slow degradation of fiber components during fermentation are main drawbacks of fungal application for nutritive improvement of WS. Yet, white rot fungi and some chemical substances such as urea, HCl and $Ca(OH)_2$ have been used as pretreatments to improve the nutritive quality of crop residues (Wanapat *et al.* 2009).

Among the family members, the *Arachniotus fungi* could be used to upgrade the nutritional value of fibrous feeds, especially in combination with SSF technology. However,optimization of the SSF culture conditionsis required to obtain the maximum benefits of fungal treatments to get improved quality WS.

Therefore, this study was planned to investigate the feasibility of using *Arachniotus sp.* in SSF system by testing various culture conditions to improve the nutritive value of wheat straw and to set-in the baseline for the subsequent production of biomass. This biomass can then be used as a feed to enhance the animal production

through better nutritional and cost effective strategies, while conserving the environment.

MATERIALS AND METHODS

Microorganism: The certified pure culture of the *Arachniotus sp.*was obtained from the stock cultures of National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad. The culture was maintained on Potato Dextrose Agar (PDA) slants and revived twice a week in PDA medium, at laboratory of Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore.

Inoculation, solid state fermentation and culture conditions: The project was conducted at the Buffalo Research Institute, Pattoki. Inoculum was prepared using fungus raised on sporulation medium. Spores of the *Arachniotus sp.* were transferred aseptically to each of three autoclaved 250 mL conical flask containing 25 mL inoculum medium. The pH of the medium was adjusted at 4.00±0.2 with 1N H_2SO_4. The flasks were incubated on the orbital shaker working at 100-120 rpm for 72h at 28^{o}C temperature.A homogenous suspension of *Arachniotussp.* was prepared with a concentration of 10^6to10^7 spores/mL, as determine by hemocytometer. The suspension was freshly prepared for each experiment and was used as an inoculum in the growth medium to investigate various fermentation conditions.The SSF substrate consisted of 5g of wheat straw (2.5-3.0 cm), weighed in erlenmeyer flask by adjusting different substrate to water ratios (1:1, 1:1.5, 1:2). The flasks were plugged with cotton wool and covered with aluminum foil to autoclave at 120^{o}C for 15 minutes. After sterilization, substrate in each flask was inoculated with1 ml of *Arachniotus sp* spores.Then flasks were maintained under stationary conditions at 28° C and monitored for eight days. All tested culture conditions were run in duplicate and all experiments were set in twice to verify the results.

Selection of optimum growth conditions for quality biomass production: In the first step, substrate to water ratios (1:1, 1:1.5, 1:2) and incubation periods (0, 2, 4, 6, 8 days) were optimized in growth medium and then these conditions were kept constant to obtain standardized salt concentrations e.g. $MgSO_4.7H_2O$ (0.025, 0.05, 0.075, 0.10%), $CaCl_2$ (0.025, 0.05, 0.075, 0.10%), KH_2PO_4 (0.05, 0.10, 0.15, 0.20, 0.25%) & urea levels (0.10, 0.15, 0.20, 0.25, 0.30%). The growth medium without any salts additionwas used as a control treatment. The pH of growth medium was adjusted at 4 using 0.1N HCl or 0.1N NaOH at 28^{o}C during the whole experiment.The dry matter and CP contents were determined according to the procedures of AOAC (2006). All culture conditions were tested in duplicate and all experiments were set in twice to verify the results. The analysis was performed at the laboratory of Department of Animal Nutrition, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki.

Statistical analysis:For analysis of incubation period and substrate to water ratio, data are presented as mean ± standard error of mean (SEM). Data were analyzed using the proc MIXED procedure of SAS (SAS Institute, 2004) according to the following statistical model:

$$Y_{ijk} = \mu + SW_i + IP_j + SW_i \times IP_j + \varepsilon_{ijk}$$

Where Yijk is the response variable, μ is the grand mean and ε is the error term

SW representsthe substrate to water ratio and IP representsthe incubation period.

While for the analysis of various salt concentrations, data are presented as mean±root mean square error (RMSE). Data were analyzed according to Completely Randomized Design using GLM procedure of SAS software. Treatments were compared with linear and quadratic polynomial contrast. The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

1: Effect of substrate to water ratio at different incubation periods on fungal growth and composition: It was observed that 1:2 substrate to water ratio facilitated the optimum fungal growth at the 4th day of incubation (Table 1, Figure 1). A significant (p<0.001) increasing trend in the CP contents of the fungal treated WS was observed with parallel increase in moisture forup to 4th day, that declined on further incubation. Our results indicated that the moisture contents in WS are a significant variable that must be controlled at the most favorable level. Too much moisture can impede the gas exchange and create anaerobic conditions, whereas inadequate water in the substrate can limit the fungal activity. Furthermore, the optimum substrate to water ratio depends on the quality, particle size and water holding capacity of the substrate (Jing and Ming, 2012). Despite the non-significant interaction, the WS treated with 1:2 substrate to water ratio showed improvement in CP (3.12%) compared to substrate treated at other ratios i.e. 1:1.5 (2.90%) and 1:1 (2.79%) at 4th day of incubation. The improved CP contents could be theresult of enrichment of fungal mycelium of fermented substrates during aerobic fermentation (Sallam *et al.*, 2007; Jonathan *et al.* 2008). Maximum CP biomass (11.20%) was also previously observed at 4th day of incubation that gradually declined thereafter in *Arachniotus sp.* treated corn cobs (El-Ashry *et al.* 2007, El-Menniaway, 2008, Asad *et al.* 2000).

2: Effect of $MgSO_4.7H_2O$: The CP contents were enhanced on increasing the concentration of $MgSO_4.7H_2O$ up to 0.05% (3.28%) compared to those of

control (3.12%) that declined significantly ($p<0.001$) on further increase in salt concentrations (Table2, Figure 2). There were quadratic ($p<0.001$) effects of $MgSO_4.7H_2O$ on CP contents of biomass. Similar to our results, $MgSO_4.7H_2O$ was previously used by other authors for biomass production by using crop residues. For the same purpose, filter pressed cake of sugar cane was treated with *Arachniotus sp*. in combination with optimized concentration of 0.015% $MgSO_4.7H_2O$ (Baig*et al*. 2002). Maximum biomass production was obtained on treatment of Eichornia and Banana peel with *Aspergillus terreus* at 0.1% $MgSO_4$, among the various concentrations (0.1 to 0.5%) tested (Jaganmohan *et al*. 2013). The increase in protein contents of the fermented substrate could be due to the effects of magnesium ions (Mg^{++}) that have been reported to favor bio-protein production by SSF (Tijani*et al.,* 2011). This uptake of Mg^{++} is ATP dependent that act as a cofactor for several enzymatic reactions, also helped in the stabilization of plasma membrane and thus played an important role in the fungal growth progression (Papagianni, 2004).

3: Effect of $CaCl_2$: The CP contents were improved by increasing calcium salts up to 0.075%. $CaCl_2$ (3.55%) compared to those of control (3.34%) that declined significantly ($p<0.05$) on further increase in salt concentrations (Table 2, Figure 3).There were linear ($p<0.001$) effects of $CaCl_2$ on CP contents of biomass.Previously, $CaCl_2$ provided about 30.13% CP at an optimized concentration of 0.075% in combination with *Arachniotus sp. and Candida utilis*to ferment rice bran in Koji fermenter (Ahmad, 2005).The increase in protein contents could be due to external Ca^{++} concentration that played a key role in fungal growth by altering ion gradients in the internal cytoplasmic compartment and thus act as a cofactor for enzymatic actions in the synthesis offungal cell wall (Chardonn*et al.* 1999).

4:Effect of KH_2PO_4: Maximum CP contents were obtained at 0.15% KH_2PO_4 (3.77%) compared to those of control (3.50%) but then initially declined and this decrease became significant($p<0.01$) for up to 0.20% increase in salt concentrations (Table 2, Figure 4).Maximum biomass protein (23.51%) was also obtained from corn stover on treatment with *Arachniotus sp.* with optimized 0.10% KH_2PO_4 (Sibtain *et al.* 2010). The potassium ions play a role in the regulation of intracellular pH, to maintain osmotic balance required for the absorption of phosphate (PO_4^{-3}) ion and thus is an important co-factor in all phases of cell metabolism (Boonyapranai, 2008)

5: Effect of Urea: The CP contents were increased parallel to increase in urea concentration for up to 0.15% (3.94%) compared to those of control (3.72%) that later-on initially declined on further addition of urea and was significantly decreased ($p<0.05$) for up to 0.25%urea (Table 2, Figure 5). The increase in CP contents might be due to interactions of urea with calcium and magnesium salts but higher levels of urea could have negative effects on fungal growth due to poor biodegradation and delignification of WS. Previous findings showed that fungal treated wheat and gram straw produced superior amount of CP combination at 3.0% urea level during 30 days of incubation in a Koji fermenter (Dahiya *et al.* 2004). While only 2 % urea gave a maximum amount (15.48%)ofprotein from corn cobs treated with *Arachniotus sp.* only during 4 days of incubation (Asad *et al.,* 2000).About 20% poultry droppingswere usedas a low cost urea source for biomass production by *Arachniotus sp.* on corn stover and the authors found a maximum amount of 23.51% of CP contents (Sibtain *et al.*, 2010).

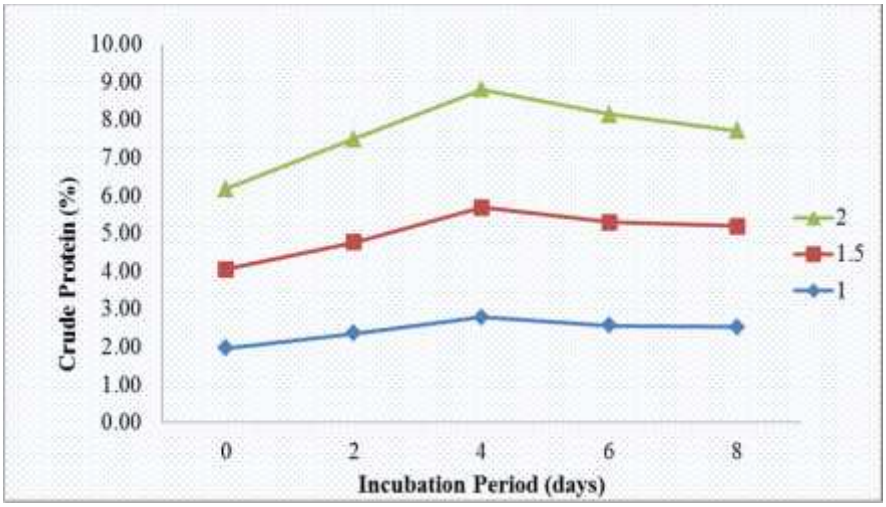

Figure 1. Change in the protein contents of wheat strawdue to *Arachniotussp.* Asaffected by differentsubstrate to water ratios and incubation days.

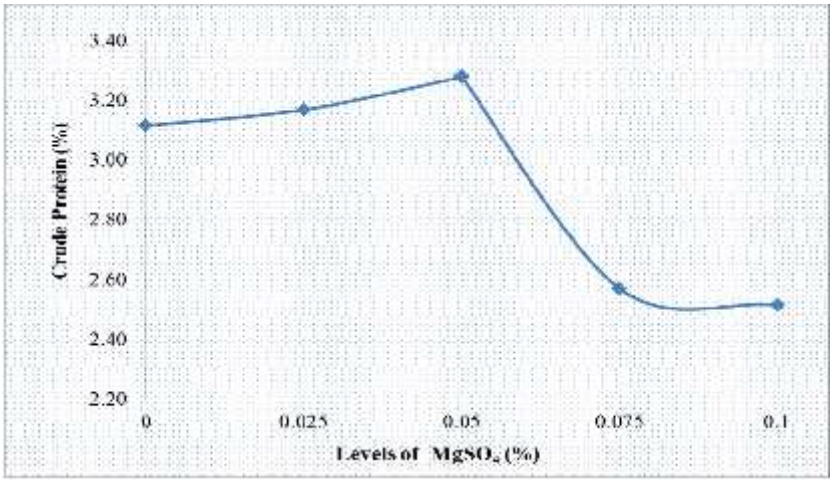

Figure 2. **Change in the protein contents of wheat straw due to *Arachniotus sp.*Asaffected by different levels of $MgSO_4$% in growth medium.**

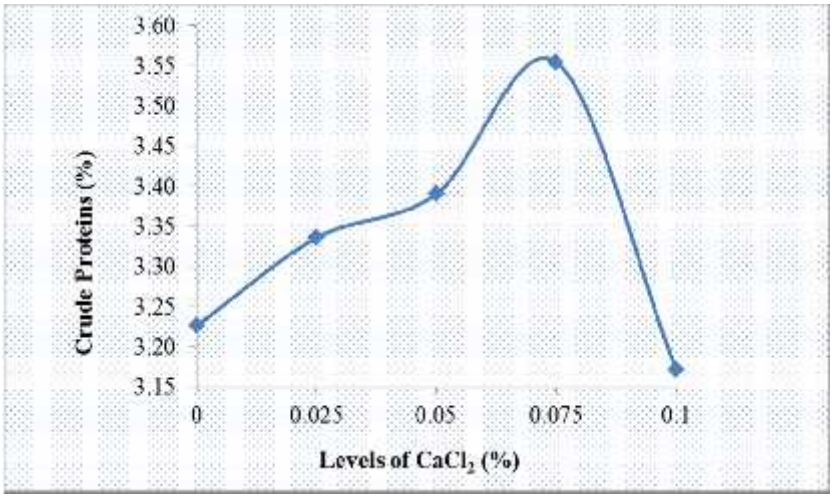

Figure 3. **Change in the protein contents of wheat straw due to *Arachniotus sp.* Asaffected by different levels of $CaCl_2$% in growth medium.**

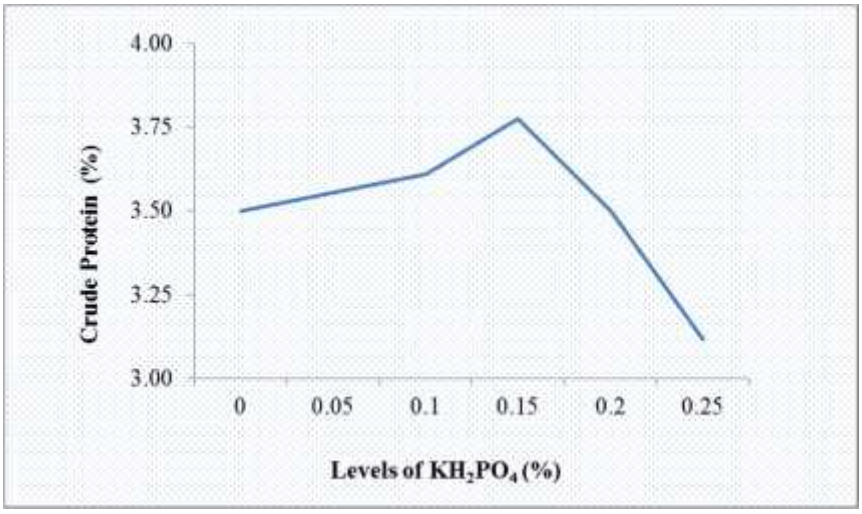

Figure 4. **Change in the protein contents of wheat straw due to *Arachniotus sp.*Asaffected by different levels of KH_2PO_4% in growth medium.**

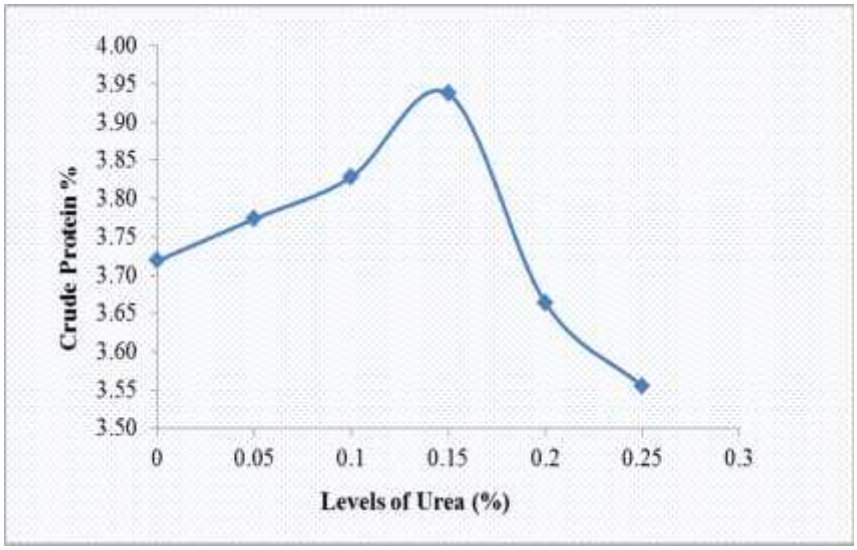

Figure 5. Change in the protein contents of wheat straw due to *Arachniotus sp.* Asaffected by different levels of Urea% in growth medium

Table 1. Crude protein contents of wheat straw incubated with *Arachniotus sp.* at varying levels of substrate to water ratios and incubation periods

Incubation period (Days)	Substrate to water ratios					
	1:1	**1:1.5**	**1:2**	**Mean**	**SEM**	***p*-value**
0	1.97	2.08	2.13	2.06 ^{d}		
2	2.35	2.41	2.73	2.50^{c}		
4	2.79	2.90	3.12	**2.94^{a}**	0.0514	<0.001
6	2.57	2.73	2.84	2.72^{b}		
8	2.52	2.68	2.52	2.57^{bc}		
Mean	2.44^{b}	2.56^{ab}	**2.67^{a}**			
SEM		0.0387				
***p*-value**		<0.001				

All values of protein contents (%) are Mean±SEM. Data represent the average of 2 samples, each analyzed in duplicate. Means containing different alphabets in superscript represent the significant difference between the treatment groups (p<0.05). Significantly higher CP values within the treatment groups are presented in bold.

Table 2. Crude protein contents of wheat straw incubated with *Arachniotus sp.* at varying levels of $MgSO_4$, $CaCl_2$, KH_2PO_4 and Urea (%) at 4th day of incubation

Salt concentrations (%)	0	0.025	0.05	0.075	0.1	0.15	0.2	0.25	Root MSE	*p*-value	Linear	Quadratic
$MgSO_4$	3.12 ^{a}	3.17^{a}	**3.28^{a}**	2.57 ^{b}	2.52^{b}	---	---	---	0.15	<0.0001	0.095	0.008
$CaCl_2$	3.23^{ab}	3.34^{ab}	3.39^{ab}	**3.55^{a}**	3.1^{b}	---	---	---	0.16	0.036	0.006	0.382
KH_2PO_4	3.50^{ab}	---	3.55^{ab}	---	3.61^{a}	**3.77^{a}**	3.50^{ab}	3.12^{b}	0.17	0.01	0.352	0.060
Urea	3.72^{ab}	---	3.77^{ab}	---	3.83^{ab}	**3.94^{a}**	3.66^{ab}	3.55^{b}	0.14	0.018	0.352	0.060

All values of protein contents (%) are Mean±RMSE. Data represent the average of 2 samples, each analyzed in duplicate. Means containing different alphabets in superscript represent the significant difference between the treatment groups (p<0.05). Significantly higher CP values within the treatment group are representedin bold.

Conclusion: It may be concluded that incubation of WS with 1:2 substrate to water ratio and 0.05% magnesium, 0.075% calcium, 0.15 % potassium and 0.15 % urea is the most suitable combination to achieve maximum CP enrichment of WS with *Arachniotus sp.* during4 days of incubation at 28^{o}C.Thus by this treatment, the nutritionally enriched WS could be used as a fibrous feed to prepare ruminant diets. This approach may facilitate the subsistence livestock farmers to change the traditional feeding management system through biotechnological

intervention. However, further studies are neededto test the shelf life, safety, palatability and digestibility of these fermented feeds for livestock consumption.

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