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Full Length Research Paper

Effect of *Trichoderma* spp. inoculation on the chemical composition and *in vitro* digestibility of wheat straw

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To evaluate the cultural ability of some *Trichoderma* isolates on wheat straw and the influence of their exogenous enzyme activities on chemical compositions as well as *in vitro* digestibility and upgrading of the nutritive value of lignocellulolytic materials, sterilized and non sterilized wheat straw were inoculated with *Trichoderma* isolates (including *Trichoderma harzianum* isolate T447, *T. hamatum* isolate T 614, *T. hamatum* isolate T625 and *T. harzianum* isolate T 969). The experimental design used in this study was complete randomized design (CRD) through factorial experiment with 2 factors (factor A = effects of sterilization, factor B = effects of *Trichoderma* spp.) in three replicates for each treatment. Effects of the *Trichoderma* isolates on the substrate neutral detergent fiber (NDF), acid detergent fiber (ADF) and pH as well as *in vitro* dry matter (DM) digestibility (IVDMD), *in vitro* organic matter (OM) digestibility (IVOMD) and *in vitro* digestible OM in DM (IVDOMD) were analyzed. The obtained results showed that sterilization of wheat straw could decrease pH, NDF and ADF ($P<0.05$) but not IVDMD, IVOMD and IVDOMD. The isolates of T447 and T969 showed higher ability in improving the nutritive value of wheat straw. Comparatively, higher reduction in pH was recorded by T614 inoculating sterilized wheat straw. The least NDF content were observed in treated non-sterilized wheat straw with T447, also treated sterilized wheat straw with T447 showed maximum reduction in NDF content. Moreover, higher change in IVDMD, IVOMD and IVDOMD were obtained by T447 grown in sterilized wheat straw substrate.

Key words: Trichoderma, wheat straw, dry matter digestibility, organic matter digestibility, digestible organic matter in dry matter.

INTRODUCTION

There is a lot of wheat straw annually produce in wheat growing belts in the world as well as in Iranian wheat growing fields. In Iran, significant amounts of wheat straw were used as a feed source for ruminants because of its abundance and low cost price. However, in many agricultural systems, a major part of wheat straw is re-incorporated into the soil. Wheat straw in comparison to forage and cereal grains has low digestibility and are voluntary taken because of the structural complexity of its plant cell wall components (Rodrigues et al., 2008). Esterified bonds made between cellulose, hemicelluloses and lignin reduce digestibility and restrict efficient utilization of the feeds by ruminal microorganisms.

In the last few years, there have been many attempts to overcome this limitation and upgrade the digestibility value of wheat straw by using chemical treatments as feed additives for ruminants such as ammonization (Barrios-Urdaneta and Ventura, 2002), sodium hydroxide as alkali treatment (Han and Anderson, 1975) and exogenous fiber degrading enzymes (Beauchemin et al., 2003; Rodrigues et al., 2008) as well as wheat straw carbohydrate consumption by fungi and bacteria species (Gangwar et al., 2003; Kansol et al., 1999; Kamra and Zadrazil, 1988). Ammonization increases crude protein

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Abbreviations: NDF, Neutral detergent fiber; **ADF,** acid detergent fiber; **DM,** dry matter; **OM,** organic matter; **IVOMD,** *in vitro* organic matter digestibility; **IVDMD,** *in vitro* dry matter digestibility; **IVDOMD,** *in vitro* digestible organic matter in dry matter.

and can improve digestibility due to the hydrolytic action of ammonia on linkages between lignin and structural polysaccharides resulting to available organic matters for ruminal microorganisms (Dean et al., 2008). Also, exogenous fibrolytic enzymes have been used to improve fiber digestibility. The use of exogenous enzymes in ruminant diet is a technology still in the developmental stage and positive effects have been recently reported. However, enzyme supplementation appears to depend on such factors as enzyme activities, type and dose of enzyme, enzyme application method, diet and animal physiological status (Giraldo et al., 2008; Colombatto et al., 2003) even the level of animal productivity (Giraldo et al., 2008; Beauchemin et al., 2003) as well as the expensive expenditure of exogenous enzyme. In the last decade, some crop residue colonizing fungi has been considered to be the most effective microorganisms in improving digestibility and nutritional qualities of ruminant feed (Akin et al., 1993). In this regards, several studies have been conducted to identify and evaluate some species of fungi for their ability in degradability and use-fulness in feed digestion. Several researchers focused on the white rotting fungi (wood decaying Basidiomycetes) such as *Pleorotus spp.* (Zadrazil, 1997) that are the most efficient fungi in depolymerization and lignin mineralization (Jung et al., 1992; Otjen et al., 1987). There are a large number of microorganisms in nature that may be useful in this regards (Falcon et al., 1995). For example, *Trichoderma* spp. have been extensively studied for their cellulase production (Nsereko et al., 2002; Domingues et al., 2000), and can grow rapidly in natural and artificial substrates; plant debris indicated the breaking down of the culture component such as polysaccharides resulting in destroying cell wall integrity (Van Wyk and Mohulatsi, 2003). *Trichoderma* spp. have clearly been established as a biological control organism against several plant pathogenic fungi (Verma et al., 2007; Howell, 2003) and have also been reported to promote plant growth (Lo and Lin, 2002). They are the most common saprophytic filament imperfect fungi in almost any soil and may be good as a biological additive to ruminants feed and an improvement to their digestibility. In this study, the cultural ability of some *Trichoderma* isolates on wheat straw and influence of their exogenous enzyme activities on chemical composition, and *in vitro* digestibility as well as upgrading the nutritive value of lignocellulolytic materials were evaluated.

MATERIALS AND METHODS

The experiment was carried out at the Animal Science Laboratory of Moghan Junior College of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran during 2008-2009. The *Trichoderma* isolates used in this study were obtained from the Plant Pest and Disease Institute, Tehran, Iran, and they include *Trichoderma harzianum* isolate T447, *Trichoderma hamatum* isolate T614, *T. hamatum* isolate T625 and *T. harzianum* isolate T969. These isolates were maintained on potato dextrose agar (PDA; BDH Ltd, UK 39 g/l) medium and stored at 4°C for further use. The isolates were recultured on PDA and incubated for one week at 25±1°C in the dark. Five discs of mycelia agar plugs obtained from the margin of each *Trichoderma* isolates (one week old growing colonies) were removed with No. 3 cork borer (5 mm diameter) and were inoculated with 1 kg sterilized wheat grain in 1 liter conical flasks (autoclaved at 121°C for 30 min twice with 24 h distance after adding 10 ml distilled water) as wheat straw inoculant's material. The control conical flasks were inoculated with 5 sterile agar discs which were obtained from sterile PDA medium. The inoculated wheat grain was incubated at 25±1°C in dark condition for two weeks. During this period, the conical flasks were mixed three times within an interval of 4 days.

Wheat straws collected from several fields were then chopped (approximately 2 cm long) and mixed completely. The obtained wheat straws were divided into two parts, one part of the wheat straw was sterilized (autoclaved in 121°C for 20 min) and the other part of was not sterilized or was in farm condition. The inoculated wheat was introduced to the wheat straw (650 with 600 g DM of sterilized and non-sterilized wheat straw at a rate of 75 g/kg DM). Water was poured over the inoculated sterilized wheat straw and non-sterilized wheat straw (750 g/kg DM) in a tube and mixed for approximately 3 min. Inoculated wheat straw were placed into the plastic containers and compressed. There were three replicates for each sterilized and non-sterilized wheat straw treatment. Treated samples were incubated in 25±1°C for 45 days. At the end of the incubation period, the treated wheat straw was removed from the plastic container. The pH of samples were freshly measured by pH meter (WPA Model CD510) according to Wilson et al. (2005) by adding 100 ml double deionized water (dd water) to 100 g of each fresh sample and shaken swiftly for 2 min before extracting the sample juice by compression. Another part of the sample was dried by exposing to free air, in shadow condition for 2 days in order to reduce excess moisture. They were then ground through 1 mm screen with a hammer mill before analyzing the neutral detergent fiber (NDF) (containing cellulose, hemicelluloses and lignin), acid detergent fiber (ADF) (containing cellulose and lignin) using the fibertec system (Foss Tecator; Model 2010) based on the method previously described by Van-Soest et al. (1991).

In vitro dry matter (DM) digestibility (IVDMD), organic matter (OM) digestibility (IVOMD) and digestible OM in DM (DOMD) were analyzed using mature Iranian-Moghani sheep (weighing 50±3 Kg, feed by alfalfa hay and 0.25 kg each of common concentrate). Rumen liquor was collected from stomach tube suction through vacuum pump according to the two-stage technique as previously described by Tilley and Terry (1963). The liquor was placed in conical flask and kept in water bath at 39°C, after straining through three layers of cheese cloth and CO_2 bubbling slightly through and then dispensed into 100 ml tubes. The experimental design used in this study was complete randomized design (CRD) through factorial experiment with 2 factors (factor A = effects of sterilization, factor B = effects of *Trichoderma spp.*) in three replicates for each treatment. The mean scores were analyzed by analysis of variance (ANOVA) and were compared by least significant difference (LSD) test at 5% significant levels with statistical analysis system (SAS) software (SAS (1985) Institute Inc., Cary, NC (USA).

RESULTS

A significant ($P<0.05$) positive correlation was observed between treated wheat straw pH and sterilization factor as well as between inoculated *Trichoderma* isolates. Also there was a significant ($P<0.05$) difference between sterilized and non sterilized substrate in reduced NDF content. There was also an observed difference between *Tricho-derma* isolates in markedly ($P<0.01$) decrease NDF

Table 1. Effects of sterilization and *Trichoderma* isolates on the pH, chemical composition and *in vitro* digestibility of wheat straw.

Items	Main effects		Interaction effects (A×B)
	Sterilization (A)	*Trichoderma* isolates (B)	
pH	***	**	*
NDF	*	**	NS
ADF	*	†	NS
IVDMD	†	**	NS
IVOMD	†	**	NS
IVDOMD	NS	*	NS

NS, P>0.10; †, P<0.10; *, P<0.05, **, P<0.01; ***, P<0.001.

Table 2. PH, chemical composition and *in vitro* digestibility of wheat straw (main effect of sterilization).

Items	Not sterilized	Sterilized	SEM
pH	9.02 a	8.46 b	0.03
NDF (g kg^{-1} DM)	830.9 a	810.0 b	7.01
ADF (g kg^{-1} DM)	480.8 a	468.0 b	4.03
IVDMD (%)	27.17 a	28.83 a	0.57
IVOMD (%)	23.86 a	25.53 a	0.67
IVDOMD (%)	22.75 a	23.31 a	0.68

Means in the same row with unlike letters are different (P <0.05). SEM: standard error of the mean.

Table 3. PH, chemical composition and in vitro digestibility of wheat straw (main effect of *Trichoderma* isolates).

Items	T 969	T 625	T 614	T 447	Control	SEM
pH	8.75 ab	8.86 a	8.64 bc	8.59 c	8.85 a	0.05
NDF (g kg^{-1} DM)	836.4 a	822.7 a	825.3 a	779.9 b	837.8 a	11.08
ADF (g kg^{-1} DM)	464.5 b	482.5 ab	486.5 a	467.7 b	471.1 ab	6.37
IVDMD (%)	29.56 ab	26.35 c	27.48 bc	30.72 a	25.89 c	0.90
IVOMD (%)	26.94 a	23.69 b	22.97 b	27.27 a	22.58 b	1.06
IVDOMD (%)	25.09 a	21.73 bc	23.11 abc	24.82 ab	20.41 c	1.08

Means in the same row with unlike letters are different (P <0.05). SEM: standard error of the mean.

(Table 1). The effect of sterilization factor in ADF content were evaluated significantly but not on various tested *Trichoderma* isolates (P<0.10). Moreover, sterilization of wheat straw showed positive effect in IVDMD and IVOMD (P<0.10) but no significant efficiency was evaluated between the mentioned factor and IVDOMD. However, different tested *Trichoderma* isolates showed significant (P<0.01) efficacy on treated wheat straw IVDMD and IVOMD as well as statistical (P<0.05) effect on their IVDOMD (Table 1). The interaction effect between these mentioned factors were evaluated and there was no significant difference in all the measured items except pH, which showed a significant (P<0.05) correlation with these different interactive effects (Table 1).

It has been found that the pH in sterilized wheat straw substrates significantly (P<0.05) decreased from 9.02 to 8.46 when compared to non-sterilized treatments as well as the NDF. ADF concentration of wheat straw decreased statistically (P<0.05) in sterilized treatments (Table 2). Based on the obtained results in Table 2, no significant differences were detected for sterilized wheat straw treatments and non-sterilized one in IVDMD, IVOMD and IVDOMD. As shown in Table 3 , treatment of wheat straw with *Trichoderma* isolates reduced signifi-cantly (P<0.05) in substrate pH but this effect did not show in T625 treated substrate which showed no statistical change in substrate pH compared to non inoculated wheat straw. Also *Trichoderma* inoculation did not affect NDF except

Table 4. PH, chemical composition and in vitro digestibility of sterilized and not sterilized wheat straw incubated by different *Trichoderma* isolates.

Items	Not sterilized					Sterilized					SEM
	T. 969	T. 625	T. 614	T. 447	Control	T. 969	T. 625	T. 614	T. 447	Control	
pH	8.93 a	9.06 a	9.05 a	8.92 a	9.13 a	8.56 b	8.66 b	8.23 c	8.26 c	8.57 b	0.07
NDF (g kg^{-1} DM)	851.6 a	836.9 ab	853.8 a	772.5 d	839.5 ab	821.3 abc	808.5 abcd	796.9 bcd	787.3 cd	836.0 ab	15.67
ADF (g kg^{-1} DM)	479.5 abc	478.4 abc	483.8 ab	475.2 abcd	487.3 a	449.4 d	486.6 ab	489.2 a	460.1 bcd	454.9 cd	9.01
IVDMD (%)	28.54 bc	26.10 c	26.62 c	28.75 bc	25.84 c	30.59 ab	26.60 c	28.33 c	32.68 a	26.06 c	1.28
IVOMD (%)	26.91 ab	23.35 bc	23.50 abc	26.27 ab	21.25 c	27.00 ab	24.04 abc	24.45 abc	28.30 a	23.90 abc	1.50
IVDOMD (%)	25.50 a	21.32 ab	23.91 a	23.94 a	19.11 b	24.70 a	22.15 ab	22.32 ab	25.70 a	21.72 ab	1.53

Means in the same row with unlike letters are different (P <0.05). SEM: standard error of the mean.

for T447 isolate which showed significant reduction in NDF concentration from 837.8 g/kg in non treated wheat straw up to 779.9 g/kg. Compared to non inoculated wheat straw, the treatment with T969 and T447 isolates reduced (P<0.05) the content of NDF. Higher change in digestibility was recorded in treated substrate with T447 isolate and T 969 isolate, respectively. IVDMD, IVOMD and IVDOMD increased markedly up to 30.72, 27.27 and 24.82%, respectively in the substrate inoculated with T447 when compared to the non inoculated wheat straw which recorded 25.89, 22.58 and 20.41%, respectively. Treated wheat straw with T969 also improved IVDMD, IVOMD and IVDOMD, significantly (P<0.05) (Table 3). According to evidence shown in Table 4, comparatively, higher reduction in pH was recorded by T614 inoculation sterilized wheat straw. The least NDF content was observed in treated non-sterilized wheat straw with T447 also treated sterilized wheat straw with T447 showed maximum reduction in NDF content. Moreover, higher change in IVDMD, IVOMD and IVDOMD were obtained by T 447 grown in sterilized wheat straw substrate.

DISCUSSION

The low nutritive value of wheat straw has been widely demonstrated (Rodrigues et al., 2008). Wheat straw consist of large amounts of cellulose and hemicelluloses; it could be an excellent energy source for ruminants, except for the high lignin content and low digestibility (Beauchemin et al., 2003) that limits its value as a feed source for ruminants. Rumen micro-organisms produce enzymes that have potential to directly hydrolyze the feed in the rumen, but the complex network formed by cellulose, hemicelluloses and lignin reduces their digestibility because of the absence of ligninolytic activity (Falcon et al., 1995; Otjen et al., 1987; Zadrazil, 1985) and this therefore restricts the efficient utilization of the feed by ruminants. Researches have been undertaken to study biological delignification for the improvement of the nutritional qualities of ruminant feed (Zadrazil, 1985). According to this, there are relatively few groups of micro-organisms able to degrade such complex compound including fungi as well as actinomycetes and other bacteria; however the most efficient lignin degrading micro-organisms are the white rot fungi (Safari-Sinegani et al., 2005). In this regards, researches have been undertaken to study the biological delignification of cell wall components of gramineous agricultural residues preferentially along with cellulose and hemicellulose by white rot fungi to improve ruminal digestibility (Jalč et al., 1997; Karunanandaa et al., 1992; Jung et al., 1992).

The aerobic fungi belonging to *Trichoderma* genus are also able to liberate several individual enzymes to hydrolyze complex plant carbohydrates such as cellulose, hemicelluloses and lignin to their monosaccharide constituent (Safari Sinegani et al., 2005). *Trichoderma* isolates are widely used in industrial applications (Van-Wyk and Mohulatsi, 2003). It is also a common source of enzymes, used in the ruminant feed as feed additives, because of its high secretory capacity

and inducible promoting characteristic. However, it is consi-dered non economical because of the high cost of commercial purified enzymes (Von-Sivers and Zacchini, 1995). Another scenario is the introduction of *Trichoderma* spp. directly into wheat straw as feed additive for degrading lignin preferentially along with cellulose and hemicelluloses. To our knowledge, *Trichoderma* species have the potential to colonize the substrate and hydrolyze complex plant carbohydrates making it possible for pre-digestion of the feed for upgrading feed intake in rumen digestion system. The enzymes effects may remain, while the inoculated feed hoard in rumen. Available data suggested that, inoculation of wheat straw with *Trichoderma* isolates could improve its nutritive value because of decreasing cell wall content and improving wheat straw digestibility (Table 2). Degradation of cell wall content indicating liberation of several enzymes into the medium consisting of cellulose and hemicellulose hydrolyzing potential of *Trichoderma* isolates were evaluated and they varied from one *Trichoderma* isolate to the other (Table 2). This finding is in general agreement with other published works that indicated that the patterns of decay and degrees of delignification vary independently with the *Trichoderma* species used and even the strain of the species (Blanchette et al., 1988).

In this study, isolates T447 and T969 grew fast on wheat straw substrate and decreased NDF and ADF as well as increased IVDMD more than other tested isolates (Table 3) indicating produce effective hemicellulolytic and cellulolytic enzymes in degrading wheat straw.

In the present study, the obtained results indicated that sterilization of wheat straw however decreased the pH as well as NDF and ADF of the substrate but did not affect the wheat straw *in vitro* digestibility treated with some *Trichoderma* isolates which could improve the feed *in vitro* digestibility. Recent studies demonstrated synergism between ruminal enzyme with exogenous enzyme in rumen indicating greater hydrolyzing activities rather than their individual activities (Morgavi et al., 2000) as well as stimulation rumen microbial number resulting to improved feed utilization (Nsereko et al., 2002). Moreover, development of *Trichoderma* in substrate depends on several factors like types of straw, storage temperature, moisture content, presence of oxygen and gaseous composition as well as other colonized microorganisms. Therefore, it seems necessary to test *Trichoderma* species and substrates to find the best situation. Further studies are needed to evaluate synergisms between *Trichoderma* enzymes and other produced metabolites in wheat straw as a substrate with ruminal enzymes and pH as well as ruminal microorganisms and their enzymes, in order to improve chemical composition and ruminal digestibility of plant material used as feed.

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