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Different *Aspergillus niger* Strains and Inoculum Levels Affect the Nutritional Composition of Olive Leaves in Solid-state Fermentation

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A R T I C L E I N F O

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A B S T R A C T

This study was conducted to investigate the effects of *Aspergillus niger* strain and inoculum level on the nutritional composition of olive leaves. The experiment had a 2×3 factorial arrangement of treatments with two *A. niger* strains (ATCC 200345 [A] and ATCC 9142 [B]) and three inoculum levels (10^4, $10^{6,}$ and 10^8). Olive leaves were milled to 2 mm and fermented in solid-state by two different *A. niger* strains and analysed for nutritional composition. Crude protein (CP) was increased linearly as the inoculum level increased. Ether extract (EE) and crude fiber (CF) were decreased linearly in A strain or quadratically in B strain with increased inoculum levels. Crude ash and nitrogen-free extract content did not differ among groups. Strain A increased neutral detergent fiber (NDF) at higher inoculum level, whereas NDF was decreased as inoculum level increased in B strain. The lowest acid detergent fiber (ADF) was obtained from the B strain or 10^6 inoculum level. The results showed that the effect of two strains on CP and ADF changed similarly with increased inoculum levels. However, two strains affected EE, CF, and NDF content differently with increased inoculum levels. The optimal situations were 10^8 inoculum level for higher CP, A10^4 or B10^4 for higher EE, B10^6 for lower CF, B10^6 or B10^8 for lower NDF, 10^6 inoculum level or B strain for lower ADF. B strain at 10^6 inoculation level can be preferred to obtain an average CP and EE content and lower CF, NDF, and ADF content.

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Introduction

The rapid increase in the world population caused a necessity to produce more animal products. With rapid development in animal husbandry, the demand for protein feed was increased around the world in the last decades. Protein sources currently used in animal nutrition will not be enough to meet the demand in animal nutrition, considering that many feed sources are also used in human nutrition. In recent years, several researchers have drawn attention to the utilization of agricultural residues.

Olive leaf is a by-product of the olive industry that emerged in the pruning and harvesting period (Molina-Alcaide and Yáñez-Ruiz, 2008). The discarded olive leaves constitute 10% of the total weight of harvested olives every year (Guinda et al., 2004). Considering that the total olive production is 21 million tons in 2017 (FAOSTAT, 2017), approximately 2.1 million tons of olive leaves are discarded each year. This causes transportation issues for the factories and also environmental problems.

Olive leaves improve egg production in quails (Christaki et al., 2011), improve immunity (Varmaghany et al., 2013) and intestinal microflora in broilers (Sarica and Urkmez, 2016), and increase antioxidant capacity in egg yolk (Botsoglou et al., 2010). Olive leaves can also improve the fatty acid profile of sheep and goat milk (Abbeddou et al., 2011a; Tsiplakou and Zervas, 2008), enhance egg yolk colour in laying hens (Cayan and Erener, 2015) and quails (Christaki et al., 2011) and reduce abdominal fat (Shafey et al., 2013) in broiler chickens. However, olive leaves can impair the growth performance of broiler chickens (Xie et al., 2016), reduce the milk yield of sheep (Abbeddou et al., 2011b), and worsen the ruminal fermentation in ruminants (Yanez Ruiz et al., 2004). These negative results may be due to the low nutritional composition of olive leaf and its anti-nutritional components (Martín-García et al., 2003).

Solid-state fermentation has become a useful method used in the utilization of agro-industrial residues in animal nutrition in recent years. Altop (2019) reported that nutritional composition of olive leaves was improved by *Aspergillus niger* solid-state fermentation. However, different *A. niger* strains are reported to having different effects on the nutritional content of olive leaves (Altop et al., 2018). Similarly, the inoculum level is one of the important factors affecting the yield in solid-state fermentation (Lakshmi et al., 2009). Altop et al. (2018) investigated the effect of different *A. niger* strains on the nutritional composition of olive leaves. However, there is a lack of information about the effects of different inoculum levels of *A. niger* strains on the nutritional quality of olive leaves. Therefore, the effects of *A. niger* strains (ATCC 200345 [A] and ATCC 9142 [B]) and three inoculum levels (10^4, 10^6 and 10^8) on the nutrient composition of olive leaves were investigated in this study.

Material and Methods

Strains and olive leaves

Aspergillus niger strains (ATCC 200345 and ATCC 9142) were obtained from American Type Culture Collection (ATCC). Olive leaves were harvested from an olive garden in Aydın province. Olive leaves were dried on a bench after collection from the trees and stored at room temperature until the fermentation process.

Solid-state fermentation

Olive leaves were divided into six treatment groups after milling to 2 mm size. They were inoculated with two different *A. niger* strains (ATCC 200345 [A] and ATCC 9142 [B]) at three inoculum levels (10^4, $10^{6,}$ and 10^8). Crude protein (CP), ether extract (EE), ash, crude fiber (CF) were determined according to AOAC (2000). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were performed according to Van Soest et al. (1991).

Statistical analysis

Differences between the treatment groups were tested using ANOVA for 2×3 factorial design. Mean differences were determined using Duncan's multiple range test. Differences were considered significant using 95% confidence limits (P<0.05). All statistical analyses were performed using SPSS (version 21.0; SPSS, Chicago, IL).

Results and Discussion

Nutritional changes in olive leaves after solid-state fermentation are given in Table 1. There was a linear increase (P<0.001) in CP with increased inoculum level (Figure 2a). Gungor et al. (2017) noted that *A. niger* can increase the CP content of the substrates may be due to produced enzymes and own mycelia. Increased CP content by fermentation was reported in the studies on *Ginkgo biloba* (Zhang et al., 2013; Zhao et al., 2013), *Moringa oleifera* (Wang et al., 2018), and *Larrea tridentata* leaves (Aguilar et al., 2008).

Ether extract content was decreased as inoculum level increased in A strain, whereas EE was decreased in B10^6 and did not differ in B10^8 compared with the B10^4 group (Figure 1, strain×inoculum level, P<0.05). Similarly, decreased ash content was reported in the studies on mango kernel (Kayode and Sani, 2008), *Terminalia catappa* fruit meal (Apata, 2011), and sour cherry kernel (Gungor et al., 2017).

Table 1. Nutritional composition of olive leaves fermented with *A. niger* strains at different inoculum levels (%, dry matter basis)

Strain	Inoculum level	CP	EE	Ash	NFE	CF	NDF	ADF
ATCC 200345 (A)	10^4	28.88	4.65^a	12.97	35.41	18.10^b	40.55^b	30.05
	10^6	30.30	3.51^{bc}	12.31	36.51	17.36^{bc}	41.91^a	29.21
	10^8	31.18	3.00^c	12.91	35.68	17.24^{bc}	42.05^a	30.95
ATCC 9142 (B)	10^4	29.34	4.20^{ab}	12.63	33.93	19.91^a	42.61^a	30.07
	10^6	29.95	3.20^c	11.91	38.82	16.12^d	39.01^c	27.29
	10^8	30.81	3.73^{bc}	12.21	36.08	17.17^c	39.09^c	29.37
SEM		0.209	0.158	0.234	0.535	0.296	0.376	0.324
Strain								
ATCC 200345		30.12	3.72	12.73	35.87	17.56	41.51	30.07
ATCC 9142		30.03	3.71	12.25	36.28	17.73	40.23	28.91
Inoculum level								
	10^4	29.11^c	4.42	12.80	34.67	19.00	41.58	30.06^a
	10^6	30.13^b	3.36	12.11	37.67	16.74	40.46	28.25^b
	10^8	31.00^a	3.37	12.56	35.88	17.20	40.57	30.16^a
P-value								
Strain		NS	NS	NS	NS	NS	**	*
Inoculum level		***	***	NS	NS	***	*	**
Strain×Inoculum level		NS	*	NS	NS	***	***	NS
Effects								
Linear		***	***	NS	NS	***	*	NS
Quadratic		NS	*	NS	*	***	NS	***

[a-d]Means in the same row without common superscripts are significantly different from each other (P<0.05), *: P<0.05, **: P<0.01, ***: P<0.001, NS: not significant, CP: crude protein, EE: ether extract, NFE: nitrogen-free extract, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, SEM: standard error of mean.

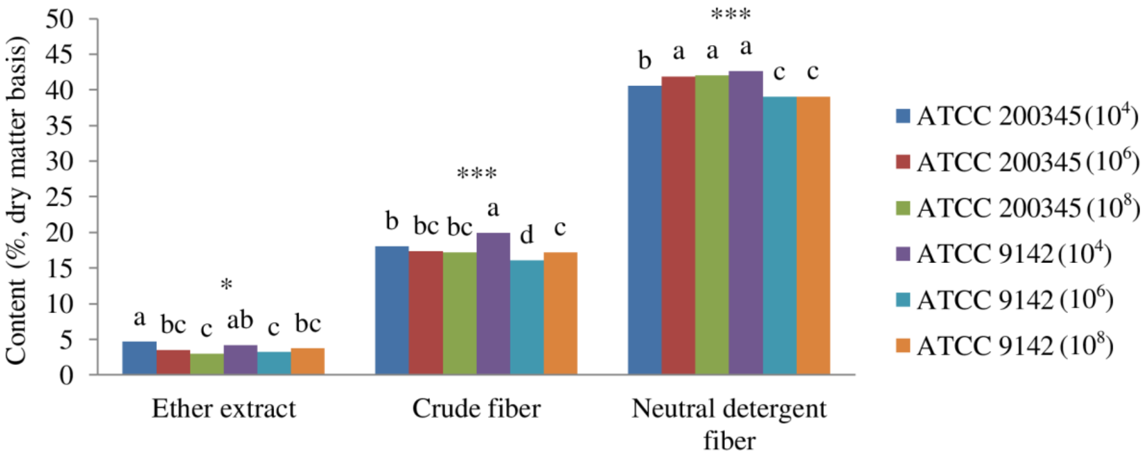

Figure 1. Interaction between inoculum level and strain on the ether extract, crude fiber and neutral detergent fiber content of olive leaves

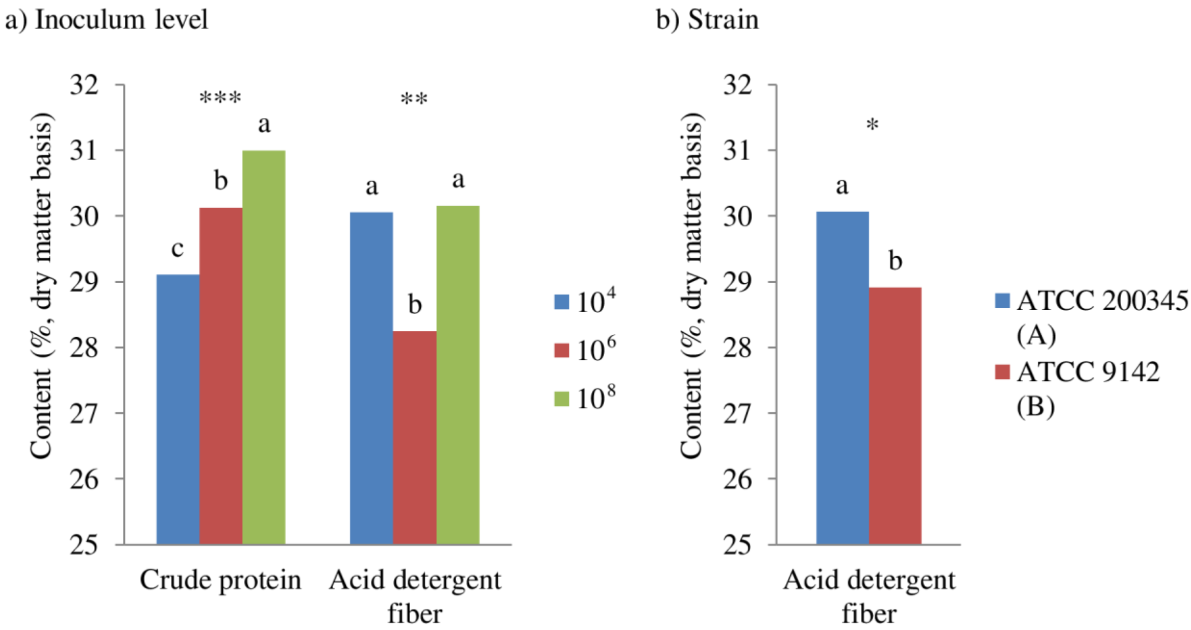

Figure 2. Effect of inoculum level (a) and strain (b) on the crude protein and acid detergent fiber content of olive leaves

The microorganisms firstly prefer soluble carbohydrates for use as a carbon source (Papagianni, 2007). Altop et al. (2018) noted that the nitrogen-free extract (NFE) content of olive leaves was decreased by solid-state fermentation. However, solid-state fermentation did not change the NFE content of olive leaves in the study. Similarly, Altop (2019) reported no change in the NFE content of olive leaves by *A. niger*.

Apata (2011) reported that fermentation decreased the ash content of *T. catappa* fruit meal. However, *A. niger* has been reported to increase the ash content of *L. tridentata* leaves (Aguilar et al., 2008) and *G. biloba* leaves (Zhang et al., 2013). In the present study, ash content was not significantly influenced ($P>0.05$) by solid-state fermentation. Similar results were obtained from the studies on palm kernel (Lawal et al., 2010) and rapeseed meal (Shi et al., 2016).

Increasing inoculum level caused a decrease in CF for B strain whereas CF was not changed by increasing level of inoculum in A strain (Figure 1, strain×inoculum level, $P<0.001$). Olive leaves in the B10^6 group had the lowest CF content among the treatment groups. *Aspergillus niger* produces cellulase enzyme and degrades the CF content of the substrates (Xie et al., 2016). Solid-state fermentation decreased the CF content of olive leaves (Altop, 2019), palm kernel (Lawal et al., 2010) and grape seed (Gungor et al., 2021).

Strain A increased NDF at a higher inoculum level, whereas NDF decreased as inoculum level increased in B strain (Figure 1, strain×inoculum level, $P<0.001$). The lowest NDF content was obtained from B10^6 or B10^8 groups. Gungor et al. (2017) noted that different *A. niger* strain affects the nutritional composition of the substrates differently, similar to the result of the present study. *Aspergillus niger* is reported to decrease the NDF content of shea nut (Dei et al., 2008), palm kernel (Lawal et al., 2010), and rapeseed meal (Shi et al., 2016).

ADF content was decreased by 10^6 and was not differ by 10^8 compared with 10^4 inoculation level (Figure 2a) and the quadratic effect was significant ($P<0.001$). B strain had lower ($P<0.05$) ADF than A strain (Figure 2b). Lakshmi et al. (2009) reported a quadratic effect of inoculum level on xylanase production, as in the ADF content in the present study. Similarly, Prakasham et al. (2006) showed that increasing inoculum level improves protease production at a certain level. But higher inoculum levels adversely affect enzyme production. Sandhya et al. (2005) also reported similar results on protease production.

Conclusion

Aspergillus niger improved the nutritional composition of olive leaves. Strains had different effects on EE, CF, and NDF with increasing inoculum levels. There was no significant difference among groups in ash and NFE content. Both strains increased CP content as the inoculum level increased. The lowest ADF content was obtained from A strain or 10^6 inoculum level. B strain at 10^6 inoculation level can be preferred to obtain the lowest CF, NDF, and ADF content and average CP and EE content.

Article Information

This study is an expanded version of the paper titled "Effect of Strain and Inoculum Level in Solid-state Fermentation on the Nutritional Composition of Olive Leaves" presented at the International Anatolian Agriculture, Food, Environment and Biology Congress held in Afyonkarahisar, Turkey on 20-22 April 2019, in line with the recommendations of the referees.

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