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Research Article

Efficacy of Seed Storage Proteins of Cereal Grains on *Aspergillus* and *Fusarium* spp.

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A B S T R A C T

The seed storage proteins contain almost half of the total proteins in mature cereal grains. They provide a great significance of nutritional value for human and livestock animals. Moreover, several other seed storage proteins possessing antifungal activity are characterized in cereal grains. In this study, antifungal activities of seed storage protein extracts from various cultivated and wild cereal species including *Triticum durum*, *T. aestivum*, *Hordeum vulgare*, *Secale cereale*, *Triticale*, *Oryza sativa*, *Avena sativa*, *A. sterilis*, and *A. fatua*, were examined on two plant pathogen fungi (*Aspergillus* spp., *Fusarium* spp.) by agar well diffusion assay. Results indicated that all protein extracts had various levels of antifungal activity; however extract of *A. fatua*, a wild type of Avena, had a superior antifungal activity on both *Aspergillus* and *Fusarium* spp. compared to the others. Extract of *S. cereale* ranged the second highest species against both fungi. In addition, extracts from Trakya (*O. sativa*), Seydişehir (*A. sativa*) and Tatlıcak-97 (*Triticale*) had a strong fungal inhibitory potential against *Fusarium* spp. As a result, protein extract from *A. fatua* may be considered as a food preservative against *Aspergillus* and *Fusarium* spp. in practice and require further identification for its components.

Keywords: Antifungal activity, Cereal grains, Seed storage proteins

Tahıllardaki Tohum Depo Proteinlerinin *Aspergillus* ve *Fusarium* spp. Üzerine Etkinliği

Ö Z E T

Tohum depo proteinleri, tahıllardaki toplam proteinlerin takriben yarısını oluşturur. Bunlar insan ve çiftlik hayvanlarının beslenmesinde önemli bir yere sahiptir. Ayrıca tahıllardaki diğer tohum depo proteinlerinin de antifungal aktiviteye sahip olduğu bilinmektedir. Bu çalışmada, kültüre edilmiş ve yabani türlerden oluşan *Triticum durum*, *T. aestivum*, *Hordeum vulgare*, *Secale cereale*, *Triticale*, *Oryza sativa*, *Avena sativa*, *A. sterilis*, ve *A. fatua* tahıllarında tohum depo proteinleri özütlerinin antifungal aktiviteleri agar well difüzyon deneyiyle iki farklı bitki patojen mantar'ına (*Aspergillus* spp., *Fusarium* spp.) karşı incelenmiştir. Sonuçlar, bütün tahıl protein özütlerinin çeşitli düzeylerde antifungal aktiviteye sahip olduğunu göstermiştir. Ancak, yabani türde olan *A. fatua* özütleri diğer türlerle kıyaslandığında *Aspergillus* ve *Fusarium* spp. üzerinde üst düzey bir antifungal aktiviteye sahip olmuştur. İkinci sırada ise *S. cereale* özütleri her iki mantar'a karşı etkili olmuştur. Buna ilaveten, Trakya (*O. sativa*), Seydişehir (*A. sativa*) ve Tatlıcak-97 (*Triticale*) özütleri ise *Fusarium* spp.'ye karşı güçlü bir mantar yok edici potansiyele sahip olmuştur. Sonuç olarak, *A. fatua* protein özütlerinin uygulamada gıda koruyucu ajan olarak *Aspergillus* ve *Fusarium* spp.'lere karşı kullanımı düşünülebilir. Ayrıca bu protein özütlerinin diğer bileşenlerinin de ileriki çalışmalarda araştırılması düşünülebilir.

Anahtar sözcükler: Antifungal aktivite, Tahıllar, Tohum depo proteinleri

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Introduction

Cereal crops are the most important agricultural commodity in the world and providing the main food sources. Among those corn, wheat and rice contribute over 70% of the total production. Other cereal crops including barley, sorghum, oats and rye are produced in lower amounts. Cereal grains contain relatively low protein content compared to legume seeds, with an average of 10-12%. Nevertheless, they provide over 200 million mt of protein for the nutrition of humans and livestock, which is about three times higher than the more protein-rich (20-40%) legume seeds (Shewry and Halford, 2002).

Besides their nutritional value as a whole grain, seed storage proteins of cereals are known to possess an important antifungal property (Macias et al., 2006). Especially, most phenolic compounds present in cereal grains have been shown to have strong antifungal activities (Dykes and Rooney, 2007). Considering the proteins in plants having antifungal activity, most of them are pathogenesis-related (PR) and in most cases their expression in plant tissues is induced by fungal or bacterial infections or following to stress conditions. Large number of PR proteins have been characterized and grouped into 14 classes based on their characteristics, such as enzymatic activity, primary structure, and biologic specificity (Loon and Strien, 1999). Similarly, cereal grains are known to express numerous PR proteins and among those PR-1, PR-2 (β-1,3-glucanases), PR-3 (chitinases), PR-5 (thaumatin-like proteins), and PR-9 (peroxidases) have been studied extensively. Class I chitinases from corn (*Zea mays*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*) and rye (*Secale cereale*), have been sequenced and biochemically characterized (Huynh et al., 1992; Leah et al., 1994; Taira et al., 2002). Moreover, it was reported that three proteins characterized as 26-kDa chitinase, 30-kDa ribosome-inactivating protein, and 32-kDa (1-3)-beta-glucanase, from barley (*Hordeum vulgare* L.) inhibited the growth of fungi (Leah et al., 1991). A recent study was reported the inhibitory effect of *A. sativa* seed storage proteins on growth of *Penicillium roqueforti*, a major contaminating species in industrial food processing (Sorensen et al., 2010). In addition, several other proteins including thaumatin-like proteins, 1,3-β-glucanase, permatin precursor, pathogenesis-related protein type 1, and chitinases of class I and II were also shown as a potential candidate for this activity (Sorensen et al., 2010).

Searching for new proteins with antifungal activity from cereal grains is an interesting topic for many practical applications, such as combating fungal plant pathogens and controlling of fungal contamination during food processing. The production of highly poisonous mycotoxins by those fungi, which contaminate food sources, is another important aspect and the prevention of fungi growth with natural antifungal agents might be an advantage for public health. Furthermore, existence of health-promotive compounds such as vitamins, minerals, and phytochemicals including phenolic compounds in whole grains should be considered as another advantage (Dykes and Rooney, 2007). However, relatively few studies were addressed the abundance of antifungal properties of proteins among different cereal grains. Therefore, the aim of this study was to search the antifungal activities of seed storage protein extracts of various cereal grains on *Aspergillus* and *Fusarium* spp. *in vitro*.

Materials and Methods

Nine cereal grain species were used in the study. Except *Triticum durum*, *Secale cereal* and *Triticale*, all other species were represented at least two different accessions or cultivars. The names of cultivars and accession numbers were; *Triticum durum* (Selcuklu 97), *T. aestivum* (Göksu-99, Gönen 98, Yüreğir-89), *Hordeum vulgare* (Konevi-98, Beyşehir-98), *Secale cereal* (Aslım-95), *Triticale* (Tatlıcak-97), *Oryza sativa* (Trakya, İpsala), *Avena sativa* (Seydişehir, Yeşilköy 1779), *A. sterilis* (CN22498 and CN22502) and *A. fatua* (CN22555 and CN25182). Wild species with Turkish origin were obtained from Plant Gene Resources of Canada (PGRC). Fungi samples were isolated as plant pathogen and kindly provided by the Adana Crop Protection Institute, Turkey. The seed storage proteins were extracted from each respective grain according to previously described method (Bal and Bay, 2010). Hulls of seeds were separated from *Avena* and *Hordeum* grains before extraction. The antifungal activity was determined by agar well diffusion assay (Schillinger and Lucke, 1989). A few loopful of *Fusarium* and *Aspergillus* spores were suspended in 1.5 ml sterile water and stored at 4°C and then 20 µl from those stocks was transferred into 20 ml of Potato Dextrose Agar (PDA) (Difco) warmed to 45°C. The samples were then vortexed and poured into sterile petri plates and kept at room temperature until agar was hardened. Subsequently, center of petri plates having agar was punchered with sterile pipet tips for application of the protein extracts. Fifty µl of the protein extraction buffer (0.125 M Tris pH 6.8, 4% SDS, 4 M urea, 5% 2-mercaptoethanol) was applied into the control plates alone, while protein extracts with the same amount of buffer from each grain sample was added on the test plates. Plates were then kept at 4°C for 1 h. After incubating at 30°C for 3 to 4 d, the observed inhibition zones around the sample applied areas were measured and recorded in mm.

Results and Discussion

The degree of antifungal activities was evaluated with the size of inhibition zones around the wells, since the same amount of fungus culture was added to each petri plates containing PDA. As depicted in Table 1, the highest inhibition zone was observed for *A. fatua* and *A. sterilis* extracts as compared to the control

where the extraction buffer was applied alone. Figure 1 represents the antifungal activities of *A. fatua* extracts on growth of *Fusarium* and *Aspergillus* spp. A previous report pointed out the inhibitory activity of *A. sativa* seed storage protein extracts on growth of Penicillium roqueforti and the specific removal of class I chitinase from extracts strongly inhibited the antifungal activity (Sorensen et al., 2010). Although class I chitinase levels of protein extracts from different cereal grains (wheat, rye, barley, avena) varied, *Avena sativa* had ten times more chitinase level than others (Sorensen et al., 2010). This was in agreement with our results, where a more antifungal activity was detected with the protein extracts of *Avena* genus. Although only seed storage protein extracts were investigated for antifungal activity in our study, several other plant parts of *Avena sativa* have been shown to possess antifungal activity. For instance, sporulation of the plant pathogens *Alternaria alternata*, *A. solani*, *Botrytis cinerea* and *Drechslera sorokiniana* was inhibited by the leaf extracts of *A. sativa* L. (Turkusay and Onogur 1998). Moreover, avenacins present in the roots of *Avena* species had also antifungal activities (Papadopoulou et al., 1999). Furthermore, *Avena longiglumis* having no avenacin was susceptible to an infection caused by *G. graminis* var. *tritici* and supporting the role of avenacin as a determinant of resistance to fungal attacks (Osbourn et al., 1994).

In addition to seed storage protein extracts from *Avena* species, strong antifungal activities were also detected for extracts from *Secale cereal* and *Triticale* samples in the present study. In addition, seed storage protein extracts of *O. sativa* (Trakya), *A. sativa* (Seydişehir) and *Triticale* (Tatlıcak-97) had an antifungal activity against the growth of *Fusarium* spp. This might indicate the level and type of antifungal seed storage protein properties of cereals changing based on variety. In accordance with this observation, certain proteins are shown to express more in some plant varieties. As an example, wheat β-1,3-glucanase gene (*TaGluD*), a fungal defense candidate, was transcribed more than 60-fold higher in leaf stage of a resistant wheat line compared to a susceptible wheat line after infection with *Rhizoctonia cerealis* (Liu et al., 2009).

The extraction procedure of seed storage proteins was successfully used for *O. sativa* samples in a previous study (Bal and Bay, 2010). Moreover, major components of rice seed storage proteins (glutelins, globulins and

Table 1. Antifungal activities of seed storage protein extracts of various cereal grains determined with agar well diffusion assay based on zone diameter (mm).
Tablo 1. Çeşitli tahıllara ait tohum depo protein özütlerinin antifungal aktivitelerinin agar well difüzyon yöntemi temeline dayalı dairesel çapla (mm) belirlenmesi.

	Fungi	
	Fusarium spp.	*Aspergillus* spp.
Cereal grains[1]	Zone diameter (mm)	
Control	26	42
Selcuklu 97[Td]	23	39
Goksu-99[Ta]	19	38
Gonen 98[Ta]	18	38
Yuregir-89[Ta]	22	39
Konevi-98[Hv]	22	37
Beysehir-98[Hv]	23	38
Aslim-95[Sc]	28	43
Tatlicak-97[Tr]	28	40
Trakya[Os]	29	41
Ipsala[Os]	26	35
Seydisehir[As]	28	38
Yesilkoy 1779[As]	26	38
CN22498[Ast]	30	42
CN22502[Ast]	30	38
CN22555[Af]	30	53
CN25182[Af]	28	42

[1]Td: *Triticum durum*, Ta: *Triticum aestivum*, Hv: *Hordeum vulgare*, Sc: *Secale cereale*, Tr: *Triticale*, Os: *Oryza sativa*, As: *Avena sativa*, Ast: *Avena sterilis*, Af: *Avena fatua*

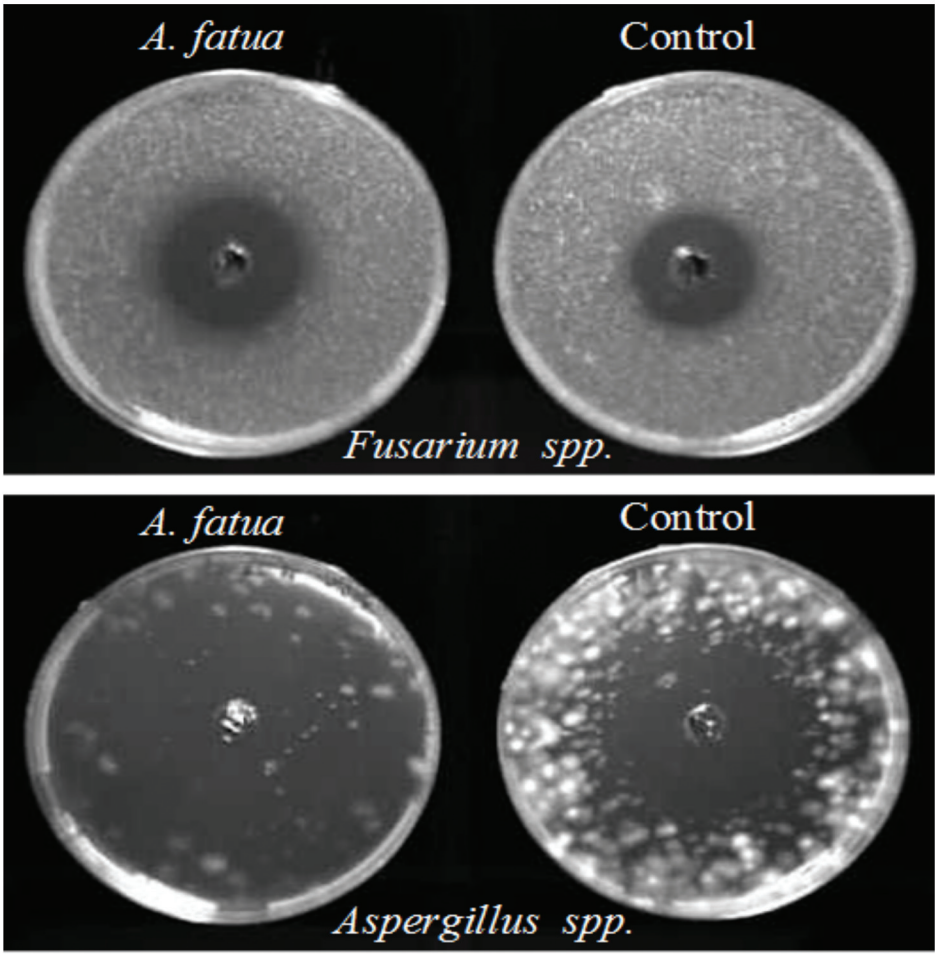

Figure 1. Effect of seed storage protein extracts on growth of *Fusarium* and *Aspergillus* spp.
Şekil 1. Tohum depo protein özütlerinin *Fusarium* ve *Aspergillus* spp.'lerin büyümesi üzerine etkisi.

prolamins) were separated on SDS-PAGE gels. Although the efficiency of the procedure was not determined for other cereal grains, an increase in inhibition levels against fungi species suggests that the procedure was more efficient for *Avena* and *Secale* samples. However, smaller inhibition zones were observed for Triticum and Hordeum extracts as compared to the control. This might indicate an antagonistic effect of some extraction components with buffer ingredients. Another explanation might be the lack of antifungal proteins in those extracts. Both explanations definitely need further investigation. Furthermore, seed hulls were separated from oat and barley samples before the extraction and antifungal activities were determined with exclusion of proteins existed in the hulls of those extracts. Therefore presence of proteins existed in hulls were not considered for antifungal activity assay.

Wild oat (*Avena fatua* L.) is the most malicious weed for cereal crops, when it invades and lowers the quality of a field crop, or competes for resources with the crop plants. Therefore it requires herbicide applications for control (Macias et al., 2010). However, results of the present study suggest the beneficial role of those protein extracts from the small grain species against undesirable fungi for other applications such as in food industry. Besides these seed proteins in cereal grains, phenolic and short-chained aliphatic organic acid constituents were also identified from wild oat (*Avena fatua* L.) (Gallagher et al., 2010). Among those, ferulic and p-coumaric acid comprised 99% of the total phenolic acids present in the seeds and most of that were found in the hulls (91%). Furthermore, malic, succinic, fumaric and azelaic acids were the dominant aliphatic organic acids detected in all seeds and chemical fractions from *A. fatua*. Phenolic acids have been shown to serve as germination inhibitors, whereas aliphatic organic acids have been linked to germination traits and protection against pathogens (Gallagher et al., 2010). In addition, all those compounds have been shown to possess anti-fungal activity in different plants (Jayasuriya et al., 2003; Abd-Alla et al., 2009).

All *A. fatua* and *A. sterilis* seeds used in the present study have been stored in the GenBank. Observing the seed storage proteins active on those fungi species indicates well preservation of the materials for a long time period. Similarly, storage conditions might affect the activity of those proteins obtained from other cereal grains. Therefore, conducting the same type of experiments with relatively fresh seed materials representing different species might be helpful for more definitive results. Furthermore, developmental control mechanisms in reproductive organs should be defined for the expression

of defensive genes. For instance, an aleurone-specific expression of barley chitinase gene has been shown to direct with an enhancer/silencer sequence (Leah et al., 1994). In addition, effects of genotype and environment on the expression level difference of the seed storage proteins should also be considered. Since a previous report showed that the environmental stress factors during grain filling affected the relative abundance of seed storage protein components in the soluble proteome of wheat dough liquor (Sancho et al., 2008).

In conclusion, results of the present study indicated strong antifungal activities of seed storage protein extracts from different cereals. *Avena fatua* extracts have the highest inhibitory activity on *Aspergillus* and *Fusarium* spp., while extracts from other *Avena* species (*A. sativa* and *A. sterilis*), *Secale cereale* and *Triticale* had also inhibitory activity on those fungi species. In addition, seed storage protein extracts from other cereal grains had also specific inhibitory activity against each fungus. The results might also be helpful for searching the possible application areas of those extracts.

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