



# Biotic interactions of cultivated mushroom and green mold disease in compost and casing soil

## Kültür mantarı ve yeşil küf hastalığının kompost ve örtü toprağındaki biyotik interaksiyonları

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### ABSTRACT

Presence of *Trichoderma aggressivum* f. *aggressivum* (green mold disease) either in compost or casing soil causes significant yield losses in button mushroom (*Agaricus bisporus*) cultivation. The aim of this study was to examine biotic interactions between *A. bisporus* and *T. aggressivum* f. *aggressivum* in compost and casing soil. In the study, strains (brown and white) of *A. bisporus* and isolates (Şem1/1 and K3) of *T. aggressivum* f. *aggressivum* were used. Experiments of compost and casing soil were conducted according to completely randomized design in factorial with four replications. Yield values of the experiments were established by weighing *sporophores* (mushrooms) in each plot. Mean yield values of each treatment were compared with control groups. Presence of the *T. aggressivum* f. *aggressivum* isolates in compost and casing soil significantly ( $P<0.01$ ) inhibited mycelial growth and fruiting body formation of both strains of *A. bisporus*. In the presence of the *T. aggressivum* f. *aggressivum* isolates in casing soil, average yield loss of the strains of *A. bisporus* was 36.41%, while it was 29.35% in the presence of the *T. aggressivum* f. *aggressivum* isolates in compost. Average yield loss of the white strain of *A. bisporus* was 49.68%, whereas it was 16.08% in the brown strain. The study revealed that presence of *T. aggressivum* f. *aggressivum* in casing soil could be much more negative influence on yield of *A. bisporus* than that of compost and brown strain of *A. bisporus* could be more resistant to *T. aggressivum* f. *aggressivum* than white strain.

**Key Words:** Compost, Casing soil, Green mold, Mushroom

### ÖZ

*Trichoderma aggressivum* f. *aggressivum* (yeşil küf hastalığı)'nın kompost ya da örtü toprağıında bulunması kültür mantarı (*Agaricus bisporus*) yetiştiriciliğinde önemli verim kayıplarına neden olmaktadır. Bu çalışmanın amacı, *A. bisporus* ve *T. aggressivum* f. *aggressivum* arasındaki biyotik interaksiyonlarının kompost ve örtü toprağındaki etkilerinin incelenmesidir. Çalışmada, *A. bisporus*'un ırkları (kahverengi ve beyaz) ve *T. aggressivum* f. *aggressivum*'un izolatları (Şem1/1 ve K3) kullanılmıştır. Çalışmadaki kompost ve örtü toprağı denemeleri tesadüf parsellerinde faktöriyel deneme desenine göre dört tekerrürlü olarak yürütülmüştür. Denemelerdeki verim değerleri her parseldeki mantar ürünlerinin tartılmasıyla belirlenmiştir. Uygulamadaki ortalama verim değerleri kontrol gruplarıyla kıyaslanmıştır. *T. aggressivum* f. *aggressivum* izolatlarının kompost ve örtü toprağıında bulunması, *A. bisporus*'un her iki ırkının misel gelişmesini ve mantar oluşumlarını önemli ölçüde ( $P<0.01$ ) engellemiştir. *T. aggressivum* f. *aggressivum* izolatlarının örtü toprağıında bulunması halinde, *A. bisporus* ırklarında ortalama verim kaybı % 36.41 olurken, *T. aggressivum* f. *aggressivum*

izolatlarının kompostta bulunması halinde ise bu deęer % 29.35 olmuştur. *A. bisporus*'un beyaz ırkında ortalama verim kaybı % 49.68 olurken, kahverengi ırkta ise verim kaybı % 16.08 olmuştur. Çalışma, *T. aggressivum* f. *aggressivum*'un örtü toprağında bulunmasının, kompostta bulunmasına göre *A. bisporus*'un verimi üzerine çok daha fazla negatif etki oluşturabildiğini ve *A. bisporus*'un kahverengi ırkının beyaz ırka göre *T. aggressivum* f. *aggressivum*'a daha dayanıklı olduğunu ortaya koymuştur.

**Anahtar Kelimeler:** Kompost, Örtü toprağı, Yeşil küf, Mantar

## Introduction

Edible mushrooms have many beneficial effects on human health with high nutritional properties such as quality of proteins, polysaccharides, unsaturated fatty acids and minerals (Ma et al., 2018). *Agaricus bisporus* (button mushroom) is a predominant species among the cultivated mushrooms (Potočnik et al., 2015). A primary problem in *A. bisporus* cultivation is green mold disease caused by *Trichoderma* species. So far, a wide range of *Trichoderma* species associated with green mold has been isolated from *A. bisporus* growing farms. However, aggressive biotypes *Trichoderma aggressivum* f. *europaeum* and *T. aggressivum* f. *aggressivum* caused substantial yield losses up to 100% in *A. bisporus* cultivation in Europe and North America, respectively (Savoie et al., 2001; Samuels et al., 2002; Sobieralski et al., 2012; Kosanović et al., 2013).

Compost and casing soil are essential components of *A. bisporus* cultivation. However, these materials might harbour aggressive and non-aggressive green mold fungi. Presence of green mold fungi either in compost or casing soil induces competition with *A. bisporus* for space and nutrient (Szczeczek et al., 2008; O'Brien et al., 2017).

Mycelia of *A. bisporus* initially grow and colonise compost and then casing soil. As a result, primordia and then fruiting bodies (sporophores) of *A. bisporus* emerge. During this phenomenon, biotic interactions between aggressive biotypes of green mold and *A. bisporus* in mushroom compost were investigated (Mamoun et al., 2000; Williams et al., 2003; O'Brien et al., 2017) but, miscellaneous

views were reported. With regard to casing soil, it was reported that various *Trichoderma* species including *T. aggressivum* could be found in diverse soils from orchards to protected areas (Abd-Elsalam et al., 2010; Błaszczuk et al., 2011; Sharma & Singh, 2014; Mirkhani & Alaei, 2015; Jiang et al., 2016). However, biotic interactions between aggressive biotypes of green mold and *A. bisporus* in casing soil have not been documented yet. Examining biotic associations during the button mushroom emergence period is crucial for determining different management strategies. Accordingly, yield losses from green mold disease could be reduced in button mushroom cultivation. The aim of this study was to examine biotic interactions between strains (brown and white) of *A. bisporus* and isolates (K3 and Şem1/1) of *T. aggressivum* f. *aggressivum* in mushroom compost and casing soil.

## Material and Methods

### *Agaricus bisporus* strains

Commercial white (192915 AG, Soc, France) and brown (Tuscan™ 860, Slyvan, Netherlands) strains of *A. bisporus* were used in the experiments. These strains of *A. bisporus* were obtained from Ersanlar Company in Korkuteli county, Antalya province.

### *Trichoderma aggressivum* f. *aggressivum* isolates

Isolate Şem1/1 [Genbank (http://www.ncbi.nlm.nih.gov) accession no: MN177935] and isolate K3 (accession no: MN177938) of *T. aggressivum* f. *aggressivum* were used from culture collection of Mycology Laboratory of Batı Akdeniz Agricultural Research Institute.

### Analysis of mushroom compost and casing soil

Samples (2 kg) were taken from the commercial mushroom compost and casing soil used in the experiments. These samples were analyzed at the Laboratory of Soil and Plant Nutrition Department of Batı Akdeniz Agricultural Research Institute (Table 1).

Table 1. Contents of mushroom compost and casing soil used in the experiments

Çizelge 1. Denemelerde kullanılan mantar kompostu ve örtü toprağının içerikleri

	Mushroom compost sample	Casing soil sample
pH	7.9	7.4
Moisture (%)	59.8	33.5
Dry matter (%)	40.2	66.5
Organic matter (%)	71.1	21.0
Ash (%)	28.9	79.0
N (%)	2.63	0.75
C (%)	41.2	17.2
C/N	15.6	22.9
P (g kg <sup>-1</sup> )	6.4	0.8
K (g kg <sup>-1</sup> )	30.6	5.6
Ca (g kg <sup>-1</sup> )	44.8	59.7
Mg (g kg <sup>-1</sup> )	6.0	30.2
Fe (mg kg <sup>-1</sup> )	1266	1467
Mn (mg kg <sup>-1</sup> )	310	543
Zn (mg kg <sup>-1</sup> )	174	32
Cu (mg kg <sup>-1</sup> )	38	26

### Preparation of inoculum

10 mL sterile distilled water per petri plate was added on the 5 day-old colonies of the *T. aggressivum* f. *aggressivum* isolates growing on potato dextrose agar (PDA). Spore suspension was filtered through a sterile cheese cloth. Conidia density were adjusted as 1×10<sup>6</sup> conidia mL<sup>-1</sup> for each isolate using a hemacytometer.

### Providing button mushroom growing conditions

The experiments were conducted in a 20 m<sup>2</sup> room in the basement of the Department of Plant Health, Batı Akdeniz Agricultural Research Institute in Antalya Province.

### Arrangement of the experiments

The experiments were set up according to completely randomized design in factorial with four replications (in treatments and controls). Experimental units consisted of plastic bags containing 2 kg compost and 650 g casing soil. Two separate experiments were established.

### Compost experiment

Inoculations of the *T. aggressivum* f. *aggressivum* isolates were performed ten days later of partly colonisation of compost by the *A. bisporus* strains. 3 mL inoculum (1×10<sup>6</sup> conidia mL<sup>-1</sup>) of each *T. aggressivum* f. *aggressivum* isolate was injected midsection of compost in the bags for each treatment. In controls, only sterile water was used. After incubation period at 25 °C for 18 days, 650 g casing soil (4 cm-thick) per bag was laid over the compost in the bag. Following this process, 25 °C temperature with 85% relative humidity were maintained for 10 days with watering the casing soil. Afterwards, the temperature was reduced one degree per day and finally room temperature was kept at 17 °C. During this period, ventilation process was performed by providing 15 minutes of oxygen from the outside per hour. Mushrooms reaching to marketing size were picked and weighed separately. Mushroom yields were obtained for each bag in the treatments and the controls.

### Casing soil experiment

In this experiment, no treatment (inoculation) was applied to compost. Mushroom growing conditions were provided as aforementioned. After laying casing soil over the compost in the bags, 3 mL inoculum (1×10<sup>6</sup> conidia mL<sup>-1</sup>) of each *T. aggressivum* f. *aggressivum* isolate was injected mid-section of the casing soil. In controls, only sterile water was used. After inoculation, mushroom growing conditions were maintained in the room as mentioned above. Mushrooms were picked and weighed per bag.

### Evaluation of the experiments

Biotic interaction between each strain of *A. bisporus* and the *T. aggressivum* f. *aggressivum* isolate was determined by comparing yield values in inoculated plots with the controls (non-inoculated). In addition, yield loss due to each *T. aggressivum* f. *aggressivum* isolate was detected through these yield comparisons for each strain of *A. bisporus*. Based on the yield loss, response of the white strain of *A. bisporus* to each *T. aggressivum* f. *aggressivum* isolate was compared with the brown strain.

### Statistical analysis

Variance analysis (ANOVA) was performed using SAS 9.1 software program (SAS Institute Inc., Cary, NC, USA). Following ANOVA, means of media, treatment, *T. aggressivum* f. *aggressivum* isolates, *A. bisporus* strains, and their interactions were compared with SAS MEANS statements with Fisher's Protected LSD<sub>0.01</sub> test option.

### Results and Discussion

Sporophores of both strains of *A. bisporus* are shown in Figure 1 in order to document the morphological differences.



Figure 1. Sporophore of the brown strain of *A. bisporus* with brown cap and short-thick stipe (on the left), sporophore of the white strain of *A. bisporus* with white cap and long stipe (on the right)

Şekil 1. *A. bisporus*'ün kahverengi ırkının kısa saplı ve kahverenk şapkalı sporoforu (solda), *A. bisporus*'ün beyaz ırkının uzun saplı ve beyaz şapkalı sporoforu (sağda)

In the variance analysis; the *T. aggressivum* f. *aggressivum* isolates, media, *A. bisporus* strains, media × *A. bisporus* strains, treatment (inoculation), and *A. bisporus* strains × treatment interactions were found significant ( $P < 0.01$ ) in the experiments (Table 2).

Table 2. Variance analysis of interactions between *A. bisporus* strains and the *T. aggressivum* f. *aggressivum* isolates in compost and casing soil experiments

Çizelge 2. Kompost ve örtü toprağı denemelerinde, *A. bisporus* ırkları ve *T. aggressivum* f. *aggressivum* izolatları arasındaki interaksyonların varyans analizi

Variation sources	Degree of freedom	Mean of squares
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates	1	6188.02**
Media (compost and casing soil)	1	37912.52**
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates*Media	1	892.69
<i>A. bisporus</i> strains	1	356557.69**
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates * <i>A. bisporus</i> strains	1	910.02
Media* <i>A. bisporus</i> strains	1	11011.02**
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates *Media* <i>A. bisporus</i> strains	1	713.02
Treatment	1	385746.02**
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates *Treatment	1	475.02
Media*Treatment	1	1598.52
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates *Media*Treatment	1	150.52
<i>A. bisporus</i> strains *Treatment	1	79137.52**
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates * <i>A. bisporus</i> strains *Treatment	1	487.69
Media* <i>A. bisporus</i> strains *Treatment	1	50.02
<i>T. aggressivum</i> f. <i>aggressivum</i> isolates *Media* <i>A. bisporus</i> strains *Treatment	1	7.52
Error	32	576.43
Total	47	

CV (%): 5.06

As a result of the inoculation of *T. aggressivum* f. *aggressivum* isolate Şem1/1, mean yield of both strains of *A. bisporus* was 485.79 g while it was 463.08 g in the inoculation of *T. aggressivum* f. *aggressivum* isolate K3. The difference was significant ( $P < 0.01$ ), indicating that *T. aggressivum* f. *aggressivum* isolate K3 was more aggressive than *T. aggressivum* f. *aggressivum* isolate Şem1/1 (Figure 2).

This also indicates that emergence of the fruiting body (sporophore) and thus yield of the strains of *A. bisporus* might change according to isolate of green mold disease. Mean yield of the strains of *A. bisporus* in the compost experiment was 502.54 g, whereas it was 446.33 g in the casing soil experiment. The difference was significant ( $P < 0.01$ ) (Figure 3).

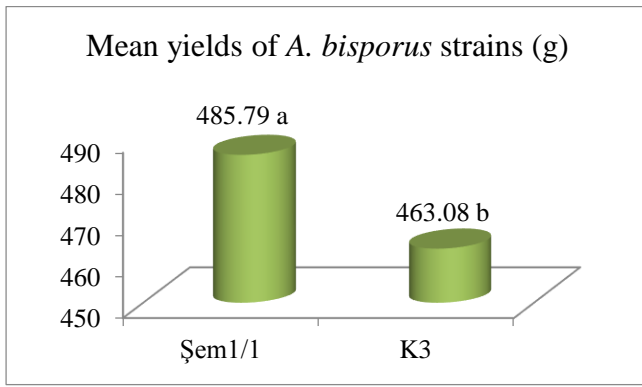


Figure 2. Mean yields of both strains of *A. bisporus* in the presence of the *T. aggressivum f. aggressivum* isolates in both experiments (LSD<sub>0.01</sub>: 13.34)

Şekil 2. Her iki denemede *T. aggressivum f. aggressivum* izolatlarının varlığında *A. bisporus* ırklarının ortalama verimleri (LSD<sub>0.01</sub>: 13.34)

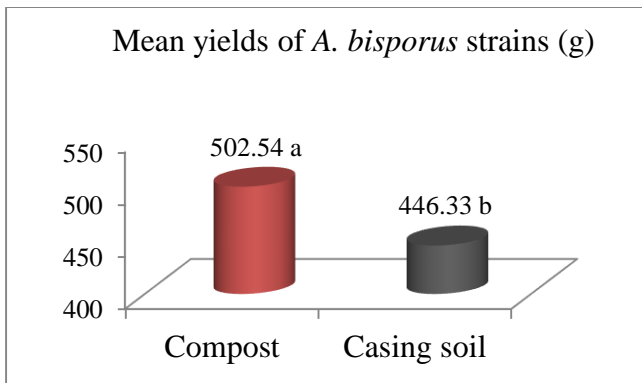


Figure 3. Mean yields of both strains of *A. bisporus* in the compost and casing soil experiments (LSD<sub>0.01</sub>: 13.34)

Şekil 3. *A. bisporus*'un her iki ırkının kompost ve örtü toprağı denemelerindeki ortalama verimleri (LSD<sub>0.01</sub>: 13.34)

This means that if the *T. aggressivum f. aggressivum* isolates exist in casing soil, the yield of strains of *A. bisporus* may be less than that of compost. This finding also shows that presence of *T. aggressivum f. aggressivum* isolates in casing soil could be more destructive on sporophore formation of *A. bisporus* than that of the compost.

Yield differences of the strains were significant (P<0.01) in the both experiments. For example, mean yield of the brown strain was 560.62 g in inoculated plots of the both experiments, whereas it was 388.25 g in the white strain (Figure 4).

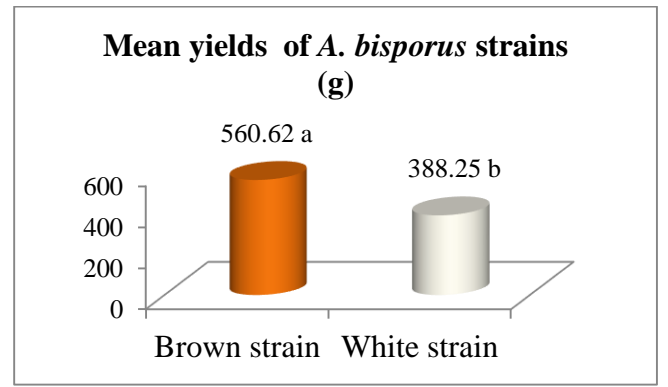


Figure 4. Mean yield of both strains of *A. bisporus* in plots inoculated with *T. aggressivum f. aggressivum* isolates (mean values of both experiments) (LSD<sub>0.01</sub>: 13.34)

Şekil 4. *A. bisporus*'un her iki ırkının *T. aggressivum f. aggressivum* izolatları ile inokule edilen parsellerdeki ortalama verim değerleri (her iki denemenin ortalama değerleri) (LSD<sub>0.01</sub>: 13.34)

This indicates that brown strains of *A. bisporus* could be affected less than the white strain in the presence of *T. aggressivum f. aggressivum* isolates either in compost or casing soil.

In the comparison of means of the both experiments, mean yield values of inoculated and the control plots were significantly (P<0.01) different from each other. In the control plots, mean yield of the strains of *A. bisporus* was 564.08 g, while it was 384.79 g in inoculated plots (Figure 5).

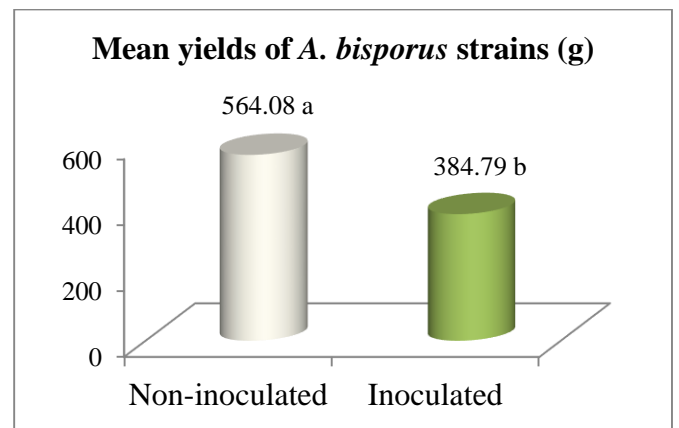


Figure 5. Comparison of mean yield of the strains of *A. bisporus* in inoculated and non-inoculated (control) plots (mean values of both experiments) (LSD<sub>0.01</sub>: 13.34)

Şekil 5. *A. bisporus* ırklarının inokulasyonlu ve inokulasyonsuz (kontrol) parsellerindeki ortalama verimlerinin kıyaslanması (her iki denemenin ortalama değerleri) (LSD<sub>0.01</sub>: 13.34)



The *T. aggressivum* f. *aggressivum* isolates caused on average 36.41% yield loss in the strains of *A. bisporus* in the casing soil experiment. However, they led to 29.35% yield loss in the strains in the compost experiment (Figure 6).

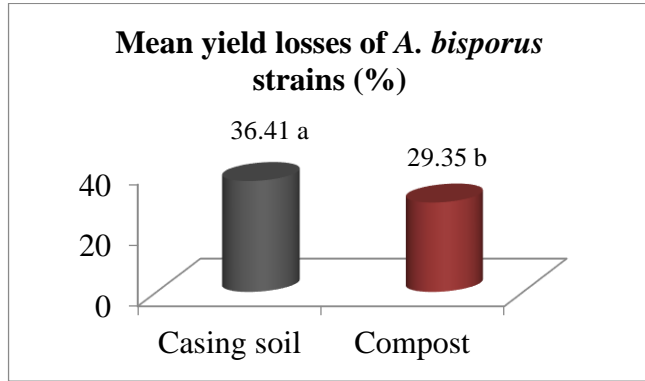


Figure 6. Compared to control plots, mean yield losses of the strains of *A. bisporus* in inoculated plots in each experiment (LSD<sub>0.01</sub>: 3.88)

Şekil 6. Kontrol parselleriyle kıyaslandığında, *A. bisporus* ırklarının her bir denemedeki inokulasyonlu parsellerdeki ortalama verim kayıpları (LSD<sub>0.01</sub>: 3.88)

In the overall evaluation of the experiments, yield losses from the *T. aggressivum* f. *aggressivum* isolates in the both compost and casing soil experiments were significantly ( $P < 0.01$ ) higher in the white strain of *A. bisporus* than the brown strain. For example, mean yield loss of the white strain in the compost and the casing soil experiments were 45.05 and 56.21%, respectively while they were 13.73 and 18.5% in the brown strain in the both experiments, respectively (Figure 7).

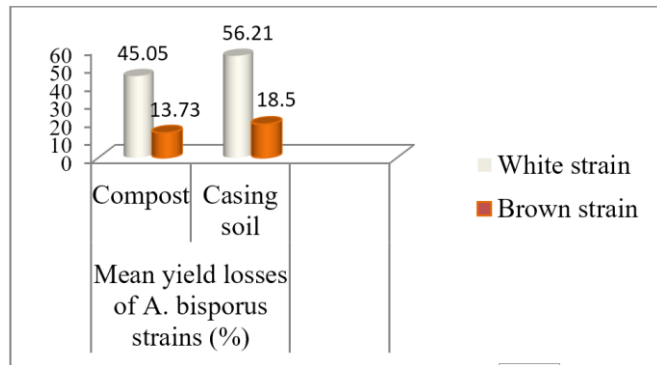


Figure 7. Comparison of mean yield losses of the strains of *A. bisporus* in inoculated plots in both experiments

Şekil 7. *A. bisporus* ırklarının her iki denemedeki inokuleli parsellerdeki ortalama verim kayıplarının kıyaslanması

Mean yield loss of the white strain of *A. bisporus* in the both experiments was 49.68% whereas it was 16.08% in the brown strain, indicating resistance of the brown strain to the *T. aggressivum* f. *aggressivum* isolates in the both experiments (Figure 8).

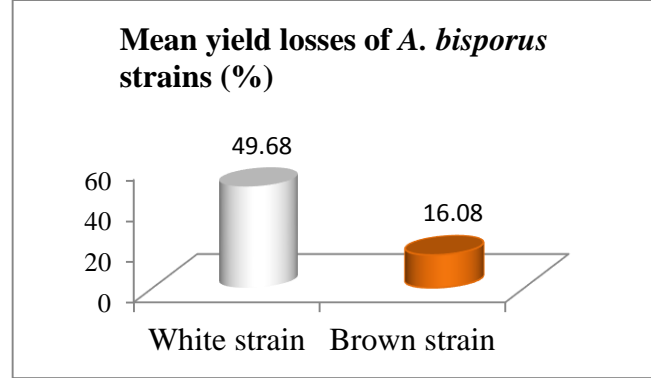


Figure 8. Mean yield losses of each *A. bisporus* strain in inoculated plots (average of both experiments) (LSD<sub>0.01</sub>: 3.88)

Şekil 8. *A. bisporus*'un her bir ırkının inokulasyonlu parsellerdeki ortalama verim kayıpları (her iki denemenin ortalaması) (LSD<sub>0.01</sub>: 3.88)

An example of the yield comparison of the brown and the white strain of *A. bisporus* for each experiment is given in Figure 9 and 10.



Figure 9. Yield of the brown strain of *A. bisporus* (on the left) and yield of the white strain of *A. bisporus* (on the right) when they were inoculated *T. aggressivum* f. *aggressivum* isolate K3 in the compost experiments

Şekil 9. *A. bisporus*'un kompost denemelerindeki kahverengi ırkı (solda) ve beyaz ırkının (sağda) *T. aggressivum* f. *aggressivum* K3 izolatu ile inokule edildiği parsellerdeki verimleri



Figure 10. Yield of the brown strain of *A. bisporus* (on the left) and yield of the white strain of *A. bisporus* (on the right) when they were inoculated *T. aggressivum* f. *aggressivum* isolate K3 in the casing soil experiments

Şekil 10. *A. bisporus*'un örtü toprağı denemelerindeki kahverengi ırkı (solda) ve beyaz ırkının (sağda) *T. aggressivum* f. *aggressivum* K3 izolatu ile inokule edildiğı parsellerdeki verimleri

Aggressive biotypes *T. aggressivum* f. *europaeum* and *T. aggressivum* f. *aggressivum* are the causal agents of green mold disease in button mushroom (*A. bisporus*) production around the world. In our study, aggressive biotype *T. aggressivum* f. *aggressivum* was used as inoculum source in the evaluation of the biotic interactions. The interactions of aggressive biotypes of green mold and *A. bisporus* in the compost were investigated by several researchers (Mamoun et al., 2000; Williams et al., 2003; O'Brien et al., 2017). But, Szczech et al. (2008) stated that in the absence of *A. bisporus* mycelium, *T. aggressivum* f. *europaeum* did not grow in compost. However, it was reported that *T. aggressivum* f. *europaeum* had the ability to grow in compost irrespective of absence or presence of *A. bisporus* (Mamoun et al., 2000; Williams et al., 2003).

With regard to *T. aggressivum* f. *aggressivum*, Beyer et al. (2000) reported that if given enough time to colonise the compost before *T. aggressivum* f. *aggressivum* introduction, *A. bisporus* could successfully colonise the compost and produce mushrooms. In our study, ten days after colonisation of compost by the strains of *A. bisporus*, the compost was inoculated with the *T. aggressivum* f. *aggressivum* isolates. This means that when the *T. aggressivum* f. *aggressivum* isolates were introduced to the compost, mycelia of the strains of *A. bisporus* had considerably colonised the compost. Even in this case, the *T.*

*aggressivum* f. *aggressivum* isolates caused severe yield (biomass) reduction in the both strains of *A. bisporus*. This finding and our results indicated that *T. aggressivum* f. *aggressivum* isolates might grow in compost and lead to significant ( $P<0.01$ ) yield losses irrespective of presence of strains of *A. bisporus* in compost. This may have been related to rapidly colonising ability of the green mold fungus. Because, quickly colonisation of compost by *T. aggressivum* f. *aggressivum* means inhibition of mycelial growth of the strains of *A. bisporus* in compost and consequently their sporophore formation. With regard to this, Beyer et al. (2000) reported that in the interactions between *T. aggressivum* f. *aggressivum* and *A. bisporus*, the ability of quickly colonising makes *T. aggressivum* f. *aggressivum* superior to *A. bisporus*. At the same time, in compost, *T. aggressivum* f. *aggressivum* produces a metabolite (3,4-dihydro-8-hydroxy-3-methylisocoumarin) inhibiting mycelial growth of *A. bisporus* (Krupke et al., 2003). In addition, compost content might be an influence on this interaction. For instance, compost with high carbohydrate but low nitrogen may promote development of green mold (Sharma et al., 2007). Other good compost traits (moisture, pH, conductivity, C/N ratio, macro and micronutrients) can also promote mycelium growth of aggressive biotypes of *T. aggressivum* f. *europaeum* and *T. aggressivum* f. *aggressivum* in compost (Beyer et al., 2000). In this context, the compost we used had good traits aforementioned (Table 1), which may have had an influence on the development of the *T. aggressivum* f. *aggressivum* isolates as well.

The brown strain of *A. bisporus* was significantly ( $P<0.01$ ) less affected by the *T. aggressivum* f. *aggressivum* isolates than the white strain in compost. This finding is consistent with the results of Anderson et al. (2001). During the interactions, the brown strain of *A. bisporus* might have abundantly generated N-acetylglucosaminidases, which makes brown strain more resilient to *T. aggressivum* f. *aggressivum* (Guthrie & Castle, 2006). In addition,

brown strains have the ability of degrading the toxin (3,4-dihydro-8-hydroxy-3-methyl isocoumarin) produced by *T. aggressivum* f. *aggressivum* more rapidly than white strains of *A. bisporus* in interactions (Krupke et al., 2003; Sjaarda et al., 2015). This may also have been an impact on the difference in responses of the both strains of *A. bisporus* in the compost in our study.

In the casing soil experiments, no inoculation was performed into compost with the *T. aggressivum* f. *aggressivum* isolates. After fully colonization of compost by the strains of *A. bisporus*, casing soil (4 cm-thick) was added to the compost. Afterwards, the *T. aggressivum* f. *aggressivum* isolates were inoculated into the casing soil. Significant ( $P < 0.01$ ) yield losses occurred in the both strains as a result of the biotic interactions between the strains of *A. bisporus* and the *T. aggressivum* f. *aggressivum* isolates in the casing soil. Because, the *T. aggressivum* f. *aggressivum* isolates rapidly colonized the casing soil and consequently mycelium growth and then primordia and sporophore formation of the both strains of *A. bisporus* did not occur. In the comparison of both experiments, the strains of *A. bisporus* formed significantly ( $P < 0.01$ ) higher yield in the compost experiment than the casing soil experiment. For example, mean yield of the strains of *A. bisporus* in the compost experiment was 502.54 g while it was 446.33 g in the casing soil experiment. This finding shows that presence of the *T. aggressivum* f. *aggressivum* isolates in the casing soil may cause significantly ( $P < 0.01$ ) higher yield loss than the compost in button mushroom cultivation. Szukács & Geösel (2018) reported that casing is an essential stage for sporophore formation and yield in button mushroom cultivation.

Casing soil with high nutritional values (e.g. organic matter) provides continuity of vegetative growth for *A. bisporus*, which does not promote of primordia formation of *A. bisporus*. In fact, nutritional stress stimulates initiating of fruiting body formation of *A. bisporus* (Choudhary, 2011). These findings indicate that *A. bisporus* completes most of the vegetative growth in the compost

while its generative growth occurs in the casing soil without much nutritional requirement. In these conditions, the biotic interaction between the strains of *A. bisporus* and the *T. aggressivum* f. *aggressivum* isolates in the casing soil may not mostly have been associated with competition for nutrients.

*Trichoderma* species could inhibit growth of other fungi using various mechanisms such as penetration of hyphae, excretion of cell wall degrading enzymes and production of toxic secondary metabolites. Therefore, inhibition of the both strains of *A. bisporus* might have been associated with the biochemical mechanism aforementioned in our study. In this context, it was reported that *T. aggressivum* f. *aggressivum* could inhibit growth of *A. bisporus* by producing antibiotic compounds (Krupke et al., 2003; Williams et al., 2003). In our study, presence of the *T. aggressivum* f. *aggressivum* isolates in the casing soil led to significantly ( $P < 0.01$ ) higher yield loss in the white strain of *A. bisporus* than the brown strain. This indicates resistance of the brown strain of *A. bisporus* to the *T. aggressivum* f. *aggressivum* isolates in the casing soil experiment as well.

## Conclusions

To our knowledge, the casing soil experiments were performed for the first time with this study. If *T. aggressivum* f. *aggressivum* isolates and strains of *A. bisporus* exist together, occurrence of biotic interactions between them is probable. As a result, presence of *T. aggressivum* f. *aggressivum* either in compost or casing soil inhibits mycelial growth and sporophore formation of *A. bisporus* and creates substantial yield losses in button mushroom cultivation. Even, presence of *T. aggressivum* f. *aggressivum* isolates in casing soil may cause significantly ( $P < 0.01$ ) higher yield loss than compost. However, brown strain of *A. bisporus* could be more resistance to *T. aggressivum* f. *aggressivum* isolates than white strain of *A. bisporus*. Considering these, in particular, presence of green mold fungi in casing



soil could be more destructive in button mushroom cultivation. Thus, management strategies should be formed in particular against green mold in casing soil in button mushroom cultivation.

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Authors' Contributions:** MA designed the study and set up experiments, İK contributed to the *in vivo* experiments, MA also analyzed the data of the study and wrote the article.

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