DETERMINATION OF FUNGAL FLORA AFTER HARVEST IN CHICKPEAS GROWN IN ADIYAMAN PROVINCE

Meltem AVAN[1*]

[1]*Adıyaman Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü, Adıyaman/Türkiye.*
ORCID ID: 0000-0002-2939-8177

**Corresponding author: meltemavan@adiyaman.edu.tr*

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ABSTRACT

With the rapid increase in the world population, food accessibility and food safety problems are increasing day by day. There are some fungal disease factors that limit production and yield in chickpea cultivation, which has a high protein content, is easy to digest, and has wide adaptability. In order to determine this fungal flora especially seen during storage, a total of thirty-four different chickpea seed samples, eight of which were taken from the chickpeas stored in open stack form in the Merkez, Kâhta and Besni districts of Adıyaman province, in 2021 and twenty-six from the products of 2022, were examined. Identifications were made with morphological and microscopic techniques together with fungal isolations using Blotter and PDA method from these seeds. At the end of the study, the fungal flora content of *Aspergillus* spp. (42%), *Rhizopus stolonifer* (29%), *Penicillium* sp. (13%), *Alternaria* spp. (7%), *Curvularia* spp. (5%) and *Trichoderma* spp. (4%) was detected. In isolations made by PDA method, *Aspergillus* spp. (39%), *Rhizopus stolonifer* (35%), *Penicillium* sp. (12%), *Trichoderma* spp. (9%), *Alternaria* spp. (3%), *Curvularia* spp. (2%) was detected. These results obtained from Adıyaman province are guiding for additional studies to be carried out for the control of chickpea microflora.

Keywords: Adıyaman, chickpea (*Cicer arietinum* L.), fungal flora, storage fungi

ADIYAMAN İLİNDE YETİŞTİRİLEN NOHUTLARDA HASAT SONRASI GÖRÜLEN FUNGAL FLORANIN BELİRLENMESİ

ÖZET

Dünya nüfusunun hızla artışı ile gıdaya erişebilirlik ve gıda güvenliği sorunları gün geçtikçe artış göstermektedir. Yüksek protein oranına sahip, sindirimi kolay, geniş adaptasyon yeteneğine sahip olan nohut yetiştiriciliğinde üretimi ve verimi kısıtlayan bazı fungal hastalık etmenleri bulunmaktadır. Özellikle depolama sırasında görülen bu fungal floranın belirlenmesi amacıyla Adıyaman iline ait Merkez, Kâhta ve Besni ilçelerine ait açık yığın şeklinde depolanan nohutlardan alınan 2021 yılında sekiz adet, 2022 yılı ürünlerinden yirmi altı adet olmak üzere toplamda otuz dört farklı nohut tohum örneği incelenmiştir. Bu tohumlardan Blotter ve PDA yöntemi kullanılarak fungal izolasyonlarla birlikte morfolojik ve mikroskobik tekniklerle tanılamalar yapılmıştır. Çalışma sonunda depo nohutlarında Blotter yöntemi ile fungal flora içeriği bakımından *Aspergillus* spp. (%42), *Rhizopus stolonifer* (%29), *Penicillium* sp. (%13), *Alternaria* spp. (%7), *Curvularia* spp. (%5) ve *Trichoderma* spp. (%4) tespit edilirken, PDA yöntemiyle yapılan izolasyonlarda da *Aspergillus* spp. (%39), *Rhizopus stolonifer* (%35), *Penicillium* sp. (%12), *Trichoderma* spp. (%9), *Alternaria* spp. (%3), *Curvularia* spp. (%2) tespit edilmiştir. Bu çalışma ile Adıyaman ilinden elde edilen bu sonuçlar nohut mikroflorasının mücadelesi için yapılacak yeni çalışmalara yol gösterici niteliğindedir.

Anahtar Kelimeler: Adıyaman, nohut (*Cicer arietinum* L.), fungal flora, depo funguslar

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the important protein-rich, easily digestible legumes belonging to the Fabaceae family (Anonymous, 1995; Rawal and Navarro, 2019). The homeland of the chickpea plant covers a region covering southeastern Turkey and northern Syria (Güneş et al., 2008). Chickpea is considered not only for human nutrition, but also as animal feed, which causes an increase in both egg and milk production (Bakr et al., 2002). Turkey is one of the largest chickpea producing country with 0.63 million tons of production on an area of 0.514 million hectares (TUIK, 2019). But there are losses in quality and yield due to many diseases, pests and abiotic stress factors.

Chickpea is infected by more than 50 diseases in many regions of the world, and most of them are known to be caused by fungi (Nene, 1980; Fakir, 1983). Many various fungal diseases such as gray mold, wilt, root and rollar rot and blight are seen in chickpeas (Bakr, 1994). Chickpeas are particularly vulnerable to many soil-borne pathogens. Among them, *Rhizoctonia bataticola* [Syn: *Macrophomina phaseolina* (Tassi) Goid] was accepted as the most important root rot disease cause threatening chickpea production (Sharma et al., 2010). *Didymella rabiei* (Kovachevski) von Arx [anamorf: *Ascochyta rabiei* (Passerini) Labrousse] affects all the above-ground parts of chickpea, but causes significant losses in seed yield and quality by causing breakage in the stem and capsule infections (Akem, 1999; Pande et al., 2005). If the environmental conditions are suitable, disease development increases and can cause yield losses up to 100% (Navas-Cortes et al., 1998; Vail and Banniza, 2009; Atik et al., 2011).

There is a mycoflora consisting of field and storage fungi in chickpeas. After harvest, field fungi gradually decrease in chickpeas and storage fungi become more dominant. Most of the storage diseases are *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp., which can cause color change in seeds and deterioration in germination (BARI, 1986).

These pathogens can cause abnormalities in the seed, reduce the germination of the seed along with other infections, and reduce the quality and market value of the product considerably. Fungi, which cause disease in chickpeas, can frequently cause disease in many different hosts around the world (Rehman et al., 2011).

Also fungi species that infect chickpeas can potentially produce mycotoxins. The main species that produce mycotoxins belong to the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria*. Mycotoxins can be highly toxic. Production of toxins; It varies depending on the contamination of the pathogen, the type of toxin, the percentage of the toxin, the general health of the plant, its age, species and possible synergistic effects between mycotoxins (Yadav et al., 2011).

Many studies have been conducted on chickpea diseases and their control, but the information about the storage of chickpea seeds is still insufficient (Dwivedi, 1989; Lal and Singh, 1997; Salam, 2004). Considering the importance and value of chickpeas, which is one of the important legumes for Adıyaman province, it has become quite necessary to investigate the fungal prevalence of stored chickpeas.

2. MATERIAL AND METHOD

In order to determine the seed infection rate, 250 g of local chickpea seeds were taken from a total of thirty-four samples from the years 2021 and 2022 from the Merkez, Kâhta and Besni

districts of Adıyaman province. In order to determine the fungal infection rates from these seeds, they were examined both macroscopically and under a stereomicroscope under laboratory conditions. Seeds were sown in PDA (Potato Dextrose Agar) and moist cell (Blotter) and the presence of disease agents was investigated.

2.1. Blotter (Moist cell) Method

In order to determine the seed infection rates, by looking at the general appearance and lesions of the seeds, 400 seeds from each group were separated to be evaluated for fungal infection according to the procedure recommended by EPPO. Then, the seeds were subjected to surface disinfection in 1% NaOCI for 3 minutes, passed through sterile distilled water twice and dried on sterile filter papers. For this purpose, 400 x 340 mm sterile filter papers were folded in accordion form in several layers, cut into plastic containers to be tested, and moistened with sterile distilled water.

The seeds were placed between folded sterile filter papers, covered with filter paper moistened with 2 layers of sterile distilled water, and incubated at 25-28 ^{o}C for 48 s. Then, the seeds were transferred to petri dishes containing PDA medium and incubated for 7-14 days (Figure 1). As a control, healthy seeds without any signs of disease on the seed coat were placed between moistened filter papers and left for incubation, then they were taken and planted in petri dishes with PDA medium. Seeds 7-14 days after incubation were evaluated for possible fungal disease agents (EPPO, 1991; ISTA, 1996).

Diagnosis of the growing fungal cultures was carried out by morphological and microscopic memory methods. Morphological characterization of fungal species was performed according to criteria recommended by current diagnostic references (Booth, 1971; Simmons, 1969, 1985; Nelson et al., 1983; Crous et al., 2006; Summerbell et al., 2011).

2.2. Potato Dextrose Agar (PDA) Method

In this method, which is used to detect deeply infecting fungi in seeds, by looking at the general appearance and lesions of the seeds, 100 seeds were separated from each group, 4 seeds in a petri dish and 25 replications to be tested. First the seeds were subjected to surface disinfection in 1% NaOCI for 3 minutes, passed through sterile distilled water twice and dried on sterile blotting papers. Then, after planting 4 seeds in the petri dish, the petri dishes were closed with parafilm and left to incubate at 25-28 ºC to grow (Figure 1). Seeds were evaluated for possible fungal disease agents 7-14 days after incubation. Diagnosis of the growing fungal cultures was carried out with morphological and microscopic diagnostic methods. Morphological characterization of fungal species was performed according to criteria recommended by current diagnostic references (Booth, 1971; Nelson et al., 1983; Simmons, 1969, 1985; Crous et al., 2006; Summerbell et al., 2011).

Figure 1. Setup of experiments with PDA and Blotter method

The contamination rates with fungal agents in the stored seeds in the experiments were calculated according to the formula below (Bora and Karaca, 1970).

$$\text{Disease Prevalence Rate (\%)} = \frac{\text{Number of Diseased Chickpea Seeds}}{\text{Total Number of Chickpeas}} \times 100$$

3. RESEARCH FINDINGS AND DISCUSSION

3.1 Fungal Agents Carried by Seeds and Their Occurrence Rates

In order to determine the fungal flora seen in chickpeas in Adıyaman province, after applying the moist cell (Blotter) and PDA method, 400 seeds in the Blotter method and 100 seeds in the PDA method were examined in total 500 chickpea seeds.

Fungi detected from 400 chickpea seed samples by moist cell (blotter) method and their percentages were determined by *Aspergillus* spp. (42%), *Rhizopus stolonifer* (29%), *Penicillium* sp. (13%), *Alternaria* spp. (7%), *Curvularia* spp. (5%) and *Trichoderma* spp. (4%) was determined.

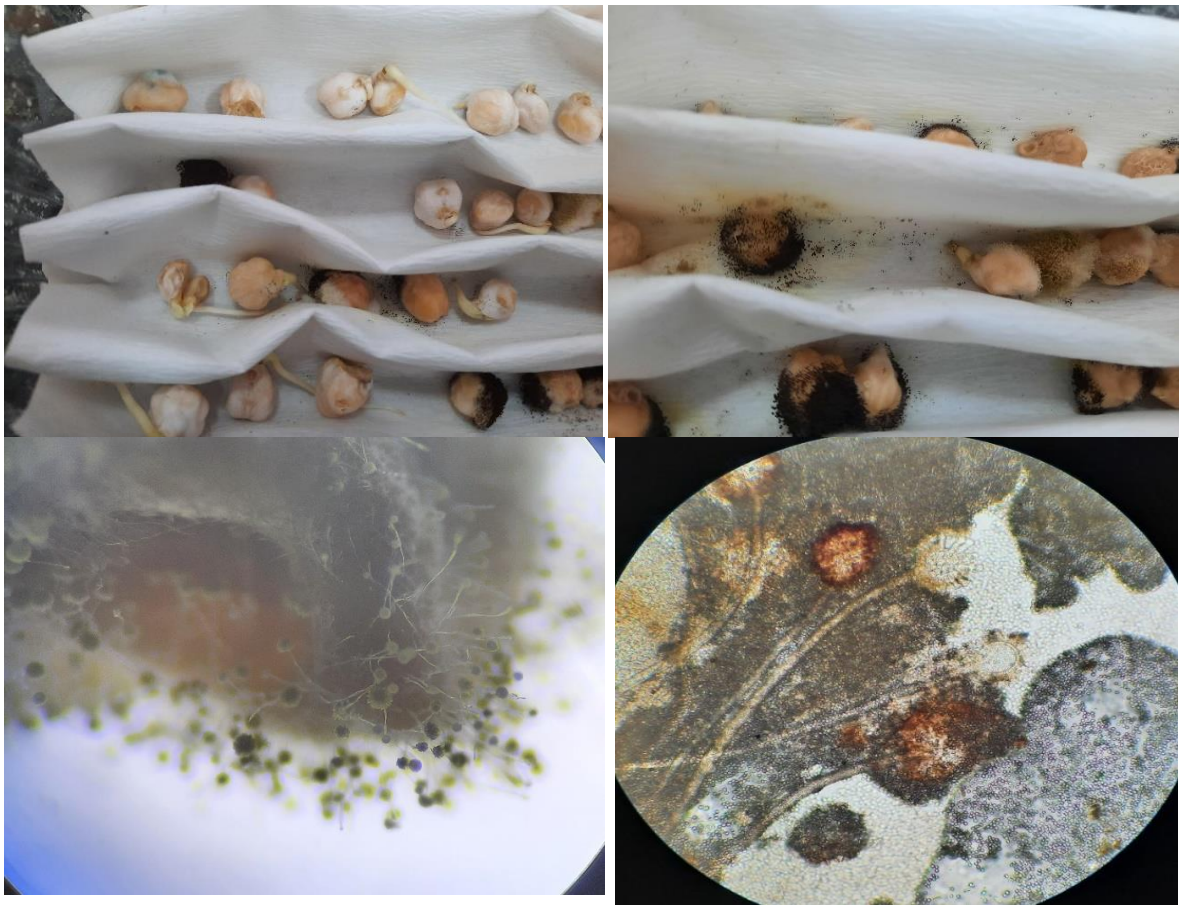

Figure 2. General view of the fungi obtained as a result of the Blotter method.

In the PDA method, 25 recurrences were made with 4 seeds in a petri dish, and as a result of the examination of 100 seeds in total, the detected fungi and their percentage were examined according to the EPPO procedure and *Aspergillus* spp. (39%), *Rhizopus stolonifer* (35%), *Penicillum* spp. (12%), *Trichoderma* spp. (9%), *Alternaria* spp. (3%), *Curvularia* spp., (2%) were found to be contaminated.

In a study conducted in 1986, *Aspergillus* spp. have been identified and these fungi have been found to significantly reduce germination in chickpea seeds (Anonymous, 1986). In the study of Dwivedi in 1989, *A. flavus, A. niger, P. oxalicum Fusarium moniliforme* were recorded in chickpea seeds (Dwivedi, 1989). Lal and Singh (1997), identified *Aspergillus, Penicillium, Alternaria, Cladosporium, Curvularia* spp. from stored chickpeas. Shamsi and Khatun found *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus nidulans, Alternaria alternata, Rhizopus stolonifer, Curvularia lunata, Penicillium* sp., and *Trichoderma viride* from stored chickpea seeds in 2016.

The fungal flora diagnoses detected in our study were found to be compatible with other studies as well. These results will shed light on the further studies required for the control of the microflora in this product.

4. CONCLUSION

Chickpea seed samples from chickpeas stored in open heap between 2021-2022 in Merkez, Kâhta and Besni districts of Adıyaman province were examined and fungal disease factors

causing damage in diseased chickpea seeds collected from these districts were determined. In this study, the highest levels of Aspergillus spp., Rhizopus stolonifer, Penicillium sp. fungi were detected. In both methods, Alternaria spp., Curvularia spp. and Trichoderma spp. found, thought at different rates. This study is important because such a study has not been carried out in post-harvest chickpeas in Adıyaman before.

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