Plant Pathology Journal

ISSN 1812-5387

science alert

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Plant Pathology Journal 4 (2): 146-149, 2005
ISSN 1812-5387

Determination of Fungi Associated with Tomatoes (*Lycopersicum esculentum* M.) and Tomato Pastes

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Abstract: Many plant pathogenic fungi are the subject of intense study because they cause disease symptoms that have a severe negative effect on the yield or quality of tomatoes (*Lycopersicum esculentum* M.) and its products. The common fungi associated with tomatoes and tomato pastes were determined and compared to get information on whether the mould content of the tomato pastes originate entirely from the tomato or from the soil. Eighteen species which belong to 7 fungal genera (*Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma*) among 250 mould isolates were determined of tomatoes and home-made tomato pastes collected from Manisa Province and its surrounding (Turkey). *Alternaria alternata* was the most prevalent fungal species. Members of *Aspergillus niger* group represent 1/6th of all the identified species. Seven of the species are the types of species that may potentially produce mycotoxins.

Key words: Mould, mycotoxin, paste, tomato

INTRODUCTION

Lycopersicum esculentum M. (Solanaceae) is one of the world's most important vegetable crops with a current worldwide fresh weight production of 80 million tones from a cropped area of about 3 million ha. The fruit improves the supply of vitamins and minerals in human nutrition[1,2]. In Turkey it is a good source of income for many farmers in both local and export trade.

Fruits and vegetables carry a natural non-pathogenic epiphytic microflora. During growth, harvest, transportation and further processing and handling the produce can, however, be contaminated with pathogens from soil, human or animal sources[3,4,6]. Fresh produce has been implicated in a number of documented outbreaks of foodborne illness particularly in Europe, Japan, United States, Canada[2] and Turkey[5,6]. Foods can be contaminated with various fungi, including *Alternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus* and *Trichoderma* species[4-7]. Therefore, the potential risks for contamination of organically grown fruits and vegetables by mycotoxin producing moulds have to be acknowledged.

As in various agricultural products, mould contamination of tomatoes starts in the fields[4,5]. Both the biological and physical damages during the harvest and transportation phases and insufficiency of hygienic conditions during tomato paste production affect the quality of the tomato paste directly by promoting the saprophyte and pathogenic mould production[5,6]. Moreover, initial high contamination levels make it difficult to reach the desired success during sterilization phase[2,6,12].

The aim of this study was to determining and comparing the common fungi occurrence in tomatoes and tomato pastes. In this way, we will have information on whether the mould content of the tomato sauces originate entirely from the tomato or from the soil. Another important point is that the ability to produce mycotoxins, play a role in food chain by tomato and tomato sauce or not.

MATERIALS AND METHODS

Collection of samples: From 2000 to 2001, 350 Tomato (from 10 tomato fields) and 30 home-made tomato paste samples were collected from the fields and markets in Manisa Province of Turkey and its surrounding. Harvest and post-harvest seasons were taken into consideration while collecting the samples.

Isolation procedure: Tomato samples were examined visually and a stereomicroscope for detecting any mould infection. Isolation and enumeration of moulds were

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performed by the method Çolakoğlu[6] using Potato Dextrose Agar (PDA) complemented with streptomycin (50 μg mL^{-1}) at 27°C. The mould intensity of the tomato pastes were determined using dilution plate technique and mould of raw material was counted using Howard mould count method[7]. Tomato products were diluted to 8% dry material and a drop was transferred on Howard slide evaluating for negative or positive for mould filaments[8]. Selected colonies of moulds on PDA were transferred from mixed culture of the plates onto respective agar plates and incubated at 27°C for 7 days. Plates containing pure cultures were stored at 4°C until further examinations.

Identification procedure: Fungal isolates were inoculated onto Malt Extract Agar (MEA), Czapek's Solution Agar (CZ) and PDA media and incubated at room temperature (25-27°C) for a period of 7 days in the dark for determining micromorphology, colony features and cultural characteristics of pure isolates. Colony diameters were measured after 7 days. Morphological observings were made with a light microscope (Model SE; Nicon) and stereomicroscope.

Penicillium L. species were identified according to standardized conditions of Pitt's monograph[9]. The identification of *Aspergillus* species was based on Raper and Fennell[10]. Other fungal species were identified according to the Domsch *et al.*[11] and Hasenekoğlu[12].

RESULTS AND DISCUSSION

Two hundred fifty mould isolates (18 species in 7 genera) were obtained from 350 tomato and 30 tomato paste samples. The distribution of the 18 species which belong to 7 fungal genera are given in Table 1. *Aspergillus fumigatus*, *A. parasiticus*, *A. terreus*, *Penicillium* sp., *P. duclauxii*, *P. roseo-purpureum* and *Trichoderma viride* species were not found in tomato pastes. Members of *Aspergillus niger* group represent 1/6th of all the identified species. The members of *Aspergillus niger* group are the most common and easily identifiable species of the genus *Aspergillus*, with its white to yellow mat later bearing black conidia[10,12]. *Alternaria alternata* was the most commonly observed species from tomato followed by *Fusarium oxysporum* and *Rhizopus oryzae*. In the tomato paste the frequency of the most common species were *Alternaria alternate*, *Aspergillus foetidus* and *Rhizopus oryzae*.

Mould intensity of the tomato paste samples were defined by dilution plate method and it was found that the maximum living mould presence is $13x10^2$ cfu g^{-1} (Fig. 1A). The same tomato paste samples were evaluated using Howard mould counting technique. Maximum rate found by this technique was 50% (Fig. 1B). This rate represents the percentage of the mouldy area number. The highest value approved by Turkish Standarts Institude is 40%[13]. There were only two samples exceeding the said value in this study. The general average was 17%.

Table 1: Identified fungal species and frequency from tomato and paste samples

	Tomatoes	Freq. (%)	Paste	Freq. (%)
Alternaria alternata (Fr.) Keissl.	+	32	+	40
Aspergillus aculeatus Lizuka	+	5	+	7
A. flavus Link	+	6	+	10
A. foetidus Thom and Raper	+	18	+	20
A. foetidus var. *pallidus* (Nakaz.) Raper and Fennell	+	10	+	3
A. fumigatus Fresen.	+	5	-	-
A. parasiticus Speare	+	6	-	-
A. terreus Thom	+	12	-	-
Fusarium oxysporum Schlecht	+	21	+	10
Mucor sp. Mich. ex Fr.	+	10	+	7
Penicillium sp. Link : Fr.	+	5	-	-
P.duclauxii Delacr.	+	7	-	-
P. frequentans Westling	+	9	+	10
P. herquei Bainier and Sartory	+	4	+	10
P. ochraceum Bainier in Thom	+	7	+	7
P. roseopurpureum Dierckx	+	5	-	-
Rhizopus oryzae Went and Prins.	+	17	+	17
Trichoderma viride Pers.	+	3	-	-

(+) Found, (-) Not found.

To our knowledge, there exist no study performed in our country on comparison of fungal flora of tomatoes and tomato pastes. Today, studies on tomato and tomato paste, around the world, concern with improving the productivity and nutrition value[14,15]. Studies on how to improve the nutrient content of tomato and thus tomato paste by using new technologies are growing in importance[16]. Also, however, the relationship between moulds and other microorganisms and tomato and tomato paste has been investigated as it is an important issue for human health.

Seven of the species isolated are the types of species that may potentially generate mycotoxins. These species and the mycotoxins they generate are: *Aspergillus flavus* (aflatoxin, sterigmatotistin and derivatives), *A. parasiticus* (aflatoxin), *A. terreus* (sitrinin, patulin), *A. fumigatus* (fumitremorgen), *Penicillium ochraceum* (okratoxin), *Alternaria alternata* (tenuazonic acid), *Fusarium oxysporum* (zearaleon, tricotesenler). By taking these into consideration, mycotoxins are introduced into the food chain via tomato fruit and products. Among the moulds isolated, the number of the species isolated from tomato samples are more than the ones isolated from tomato paste samples. Exposure to sun during tomato paste process, which is in a way treatment with heat, plays an

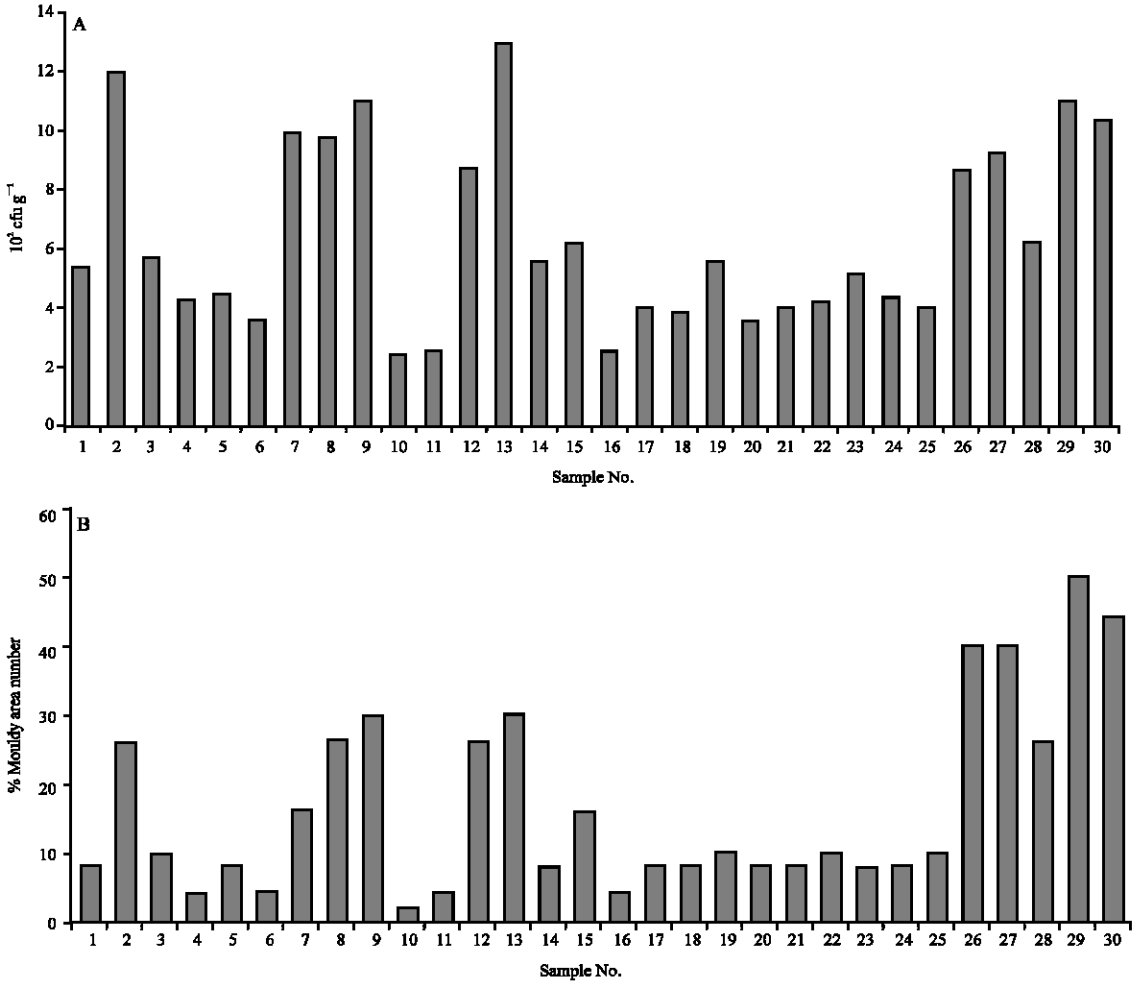

Fig. 1: Mould intensity of paste samples, A) Dilution plate method; B) Howard mould count technique

important role here. Because some mould filaments are not resistant to heat and UV[17]. When the risks caused by mycotoxins are taken into account, it can be seen that such studies will be being performed for a long time.

The main source of the mould contamination in either tomato or tomato sauce is soil[18,19]. However, another issue which plays role in the intensity of moulds in tomato and tomato paste is the harvest season. As a general rule, if 70% of the fruits have become red, the fruits should be harvested. However, as the producers wish for more red fruits, they wait for a couple of more days and this causes mould contamination. Moreover, mould contamination rate increases if harvest can not be carried out for any reason such as inappropriate weather[20] .

Fungal flora of the tomato fruits grown in our region and that of the tomato pastes produced using these tomatoes have a similarity. Mould intensity of the final product, tomato paste, depends on the pains taken during growing and harvesting the tomatoes. Harvesting time, watering period and method should be well adjusted to avoid mould contamination. Separate of moldy fruits, quick transport, keep in cold, hygiene on process were also important during transportation, storage and processing of the tomato fruits after harvesting helps to increase the quality of the final product.

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