Post harvest fungi associated with *Solanum lycopersicum* (Tomato) fruits collected from different markets of Mumbai

Benita B. Rodrigues & Umesh B. Kakde*
Department of Botany, The Institute of Science, 15-Madam Cama Road, Mumbai-32, MS, India
***Corresponding Author:** Umesh B. Kakde

Abstract

Solanum lycopersicum L. (Tomato) is an important vegetable crop in India and is consumed in diverse forms including raw, ingredient in several dishes, sauces, salads, drinks etc. It is considered as a vegetable for culinary purposes. China and India is the largest producer of this crop. Currently tomato has a higher consumption rate in more developed countries and is often referred to as a luxury crop. The fruit is rich in lycopene, which has beneficial health effects.

Many fungi associated with this fruit after harvest which is responsible for degradation and deterioration. Hence, the study was conducted for the isolation, characterization and identification of fungi associated with fresh and spoiled tomato fruits collected from different markets of Mumbai. Whole fruit were kept in sterile moist blotter chamber for the growth of associated fungi. Also the small piece from infected fruit skin tissues cut with sterile scalpel and placed on to sterile Potato dextrose agar and Czapek' s dox agar in Perti dishes and incubated at 28 ±1°C for 4 to 5 days. The mycelia of fungi were fixed in lactophenol and or cotton blue on to glass slides and identified up to species level.

The major fungi isolated from tomato fruits were *Alternaria alternata, Aspergillus niger, Aspergillus flavus, Colletotrichum sp., Rhizopus sp.* and *Fusarium oxysporum*, were the most common and frequently isolated fungi. While, *Botrytis cinerea* and *Penicillium digitatum*, *Penicillium chrysogenum*, *Phoma sp., Cladosproium sp., Geotrichum candidum* were the least fungi isolated during the period of investigation. Spoiled tomato fruits collected from market places had the highest occurrence of fungal isolates than the fresh fruits collected from the markets same market.

KEYWORDS: Post harvest, Fungi, *Solanum lycopersicum*, Vegetable crop, Lycopene, Health effects, Market

Introduction:

Tomato is rich in vitamins, carbohydrates, proteins, fats and potassium (Talvas et al., 2010). In recent years, tomato has attracted the attention due to the anticarcinogenic and antioxidant property of lycopene and ascorbic acid contents. Lycopene being efficient quencher of singlet oxygen and free radicals provides protection against a broad range of epithelial cancers (Di Mascio et al., 1991). The production of the normal red colour of ripe fruit is due to the destruction of chlorophyll and the extensive accumulation of the carotenoids (lycopene, β -carotene) while the chloroplasts are transformed into

chromoplasts. In developing countries like India, tomato is becoming more important part of the food basket; hence the farmers aim to increase quantity than quality of the produce.

World vegetable and fruit losses can depend upon soil and air borne pathogens. Fungal pathogens are considered to be one of the highly damaging agents that cause the reduction of tomato production and post harvest losses. Post-harvest diseases are responsible for significant loss of vegetable and fruit production (Kakde et al., 2012). Post harvest losses are estimated to range from 10 to 30 % per year despite the use of modern storage facilities and techniques (Harvey, 1978). In developing countries like India the postharvest losses are more severe due to inadequate storage and transportation facilities (Sharma et al., 2009).

A significant proportion of post-harvest losses are also due to post harvest diseases caused by fungi and bacteria. Losses caused by post-harvest diseases are greater than generally realized because the value of fresh fruits and vegetables increases several fold while passing from the field to the consumer (Eckert & Sommer, 1967). Post harvest handling, weak storage practices, transportation and improper marketing are seriously affecting the quality of tomatoes. Improper handling and storage practices may responsible for the degradation and deterioration of this fruit. Fruits and vegetables contaminated with mycotoxin producing fungal species such as *Aspergillus*, *Fusarium* etc. are dangerous for human health, because they produce mycotoxin which is carciniogenic in nature (Willie, 1978; Bullerman, 1979; Kakde et al., 2017).

Fungi are more commonly found attacking fruits and vegetables as postharvest pathogen. Spoilage is refers to any changes in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accomplished by alternations in taste, smell, appearances or texture. These microbes render fresh fruits and vegetables unfit for human consumption by causing their deterioration and leading to reduction in quality, texture, off flavor development and loss of nutrients (Lund, 1992).

Though much of research work has been conducted abroad and in India on various aspects of tomato fruit rots, yet not much is known about the different aspects of post harvest fungal diseases become a serious constraint. The data on post harvest disease of tomato is scanty and no data available on the post harvest diseases from Mumbai region where the vegetables and fruit commodities come from all over Maharashtra and neighboring states. The weather condition in Mumbai is favorable for the growth of many microbes due to hot and humid conditions.

Materials and Methods:

Survey of postharvest diseases of tomato fruit: For the study of post harvest diseases of tomato a regular survey was conducted in Mumbai. Samples of fresh and infected fruits were randomly collected from 4 different markets in Mumbai every month (viz., Dadar, Ghatkopar, Fort and Andheri). Ten samples were collected from each market. Fruit surface showing symptoms of fungal infections were cultured to identify the associated pathogens. The disease incidence was recorded by counting number of fruits showing fungal infection (rotting symptoms) and calculated by using formula:

$$\text{Percent disease incidence} = \frac{\text{No. of infected fruits} \times 100}{\text{Total No. of fruits observed}}$$

Furthermore, the fresh, diseased, infected fruit samples were collected from different markets of Mumbai. The fresh and spoiled or discarded tomatoes including mechanically wounded or bruised, diseased etc. were collected in pre-sterilized polythene bag and brought back into the laboratory for further investigations. The unwashed samples were kept in pre-sterilized moist blotters chambers separately. The samples were incubated at 28°C for 3 to 5 days to allow the growth of fungi associated with it.

Also the diseased or decayed fruits sample was examined for the fungi by taking surface issues from the infected part or margins. Small sections of infected fruit were cut and surface sterilized individually in 2% sodium hypochlorite for 1 min and rinsed twice in sterile distilled water to remove the tresses of $HgCl_2$ and then aseptically transferred to Petri dishes containing Potato dextrose agar (PDA) and CZ. The plates were incubated for the growth and sporulation of fungi. Fungi were carefully isolated and the slides were prepared in lactophenol cotton blue mounting on the glass slide. The microscopic slides were covered with a cover slip and were examined under the microscope for morphological examination. The culture thus obtained was observed under the microscope for various cultural and morphological characters viz., mycelial growth, shape, size, colour and microscopical characters of the fungus so as to identify the pathogen. On the basis of cultural and morphological characteristics the fungal pathogens were identified with the help of descriptions given in standard literatures (Ainsworth, et al. 1972; Barnett, 1960; Ellis, 1971; Ingold, 1974; Gilman, 1957; Smith, 1969).

Results and Discussion:

Fungal rot is world-wide problem as it has been reported almost in all parts of the world. According to Sokhi and Sohi (1982), the destructive pathogen causing fruit rots on tomato is reported from the countries where moisture is plentiful and temperatures are moderate, favoring its development. Losses due to fruit rot diseases are associated with a number of factors such as commodity type, cultivar susceptibility to the disease, the environmental events (temperature, relative humidity, atmosphere composition etc.) and/or ripeness stages.

Every vegetables and fruits have different resistance capacity to decay and infections but they are most resistant when they are dry. Mature green tomatoes are more resistant to most decay pathogens than are ripe fruit. Over-ripe fruit may be attacked by organisms, such as *Penicillium* spp., that normally are not considered decay pathogens of tomatoes (Jerry et al., 2017). In this investigations species of *Aspergillus, Fusarium, Alternaria* are the major fungi associated with the post harvest decay of the tomato.

In this investigation the molds like *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Colletotrichum gloeosporioides* and *Fusarium oxysporum* were most prevalent among all isolates. Temperature and humidity are important factor, which affect the vegetable fruits and provides medium for the growth of fungal pathogens. This is in agreement with the findings of Kutama et al. (2007) reported several fungal pathogens associated with the tomatoes. *Alternaria* rot has been considered a common diseases and causes huge losses to tomatoes thus making tomatoes unfit for consumption (Douglas 1922). The magnitude of post-harvest losses always vary from one country to

another country and one season to another and even one day to another (Mujib et al., 2007). The climate of Mumbai is hot and humid which is favorable for the fungal growth.

Vegetable and fruit rots are generally caused by opportunistic fungi that normally live on packaging materials and plant debris. However, these opportunists can infect tissues that are wounded or exposed to air. These microorganisms are ubiquitous in the nature and can infect the vegetables and fruits. Mechanical injuries on the fruit surface that occur during harvest and post harvest activities like transportation, storage, marketing and handling etc provide the site for fungal infections. Internal bruises may occur during harvest, transportation and storage and fungi can colonize internally and deteriorate the quality and decay of vegetable and fruits.

Srivastava et al. (1966) gave the systematic account of fungal diseases of tomatoes during storage. They reported many fungi viz., *Alternaria tenuis, Colletotrichum dematium, Cladosporium fulvum, Fusarium roseum, Malustela aeria, Myrothecium roridum, Oospora lactis f. parasitica, Phoma* sp. and *Rhizopus nigricans. Similar results were also registered in the present investigation.* Osakwe et al. (2010) identified three species of fungi viz., *Fusarium moniliforme, R. stolonifer* and *Geotrichum candidum* that were responsible for tomato rot and reported all the fungi were found to be pathogenic on different varieties of tomatos. They also tested severity of infection of fungal isolates on the three varieties and reported 100 per cent severity of infection by *R. stolonifer* and 92 and 90 per cent severity by *G. candidum* and *F. moniliforme* respectively.

In the present investigation *Aspergillus niger* and *A. flavus* showed 95% and 67% association with the fruit samples collected from all the different markets. Followed by *Fusarium oxysoprum* (49%), *Alternaira alternata* (53%), *Colletotrichum gloeosporioides* (35%) and *Rhizopus stolonifer* (20%) respectively. The different fungal species such as *Penicillium, Cladosporium, Geotrichum, Verticillium, Rhizoctonia solani* also found associated with the fruit samples with varying frequencies. Frequency of occurrence of fungal isolates from different locations is given in Table 1 and Per cent contribution of major fungal species associated with tomato fruit in different markets of Mumbai is depicted in Fig#1. The important pathogens causing post harvest diseases of tomato are *Alternaria, Aspergillus, Rhizopus* etc., which make the fruit not only to lose its attractive appearance but also make them to become soft and watery (Ratnam and Nema, 1967).

Ogaraku et al. (2010) carried out work on storage decay of tomato and vitamin C content of infected fruits in Nigeria in four locations and they reported that out of 48 samples of tomatoes 34 samples had fungal associations. The species of fungi isolated and identified from deteriorated tomatoes were *Aspergillus niger, A.flavus, Alternaria alternataani* and *Fusarium oxysporum*. Abdel-Mallek et al. (1995) while studying the microflora of tomato fruit from Assiut (Egypt) reported that *A. alternata, Rhizopus stolonifer* and *A. niger* were abundant with their maximum occurrence at 53, 36 and 25%, respectively. Similar results were obtained in the present investigation. It was also reported that the vitamin C content of uninfected tomato fruits was more than that of infected tomato fruits. It shows that the fungal infection can reduce the quality of the fruits as well as the important nutrients also depleted due to fungal attack.

Kakde et al., 2001 and 2012 carried out an extensive survey of vegetable and fruit market environment. They reported *Aspergillus niger*, *A. flavus*, *Penicillium chrysogenum*, Curvularia *sp.*, *Alternaria sp.*, *Fusarium sp*. The fungi like *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Alternaria* etc. were the most frequent associated fungi isolated from the vegetable and fruits. These fungi were most prevalent in the air of market environment and also found to be responsible for most of the decay of the vegetables during storage. Hence, there is probably a cyclic relationship existing between the prevalence of fungal bioaerosols and spoilage diseases in market environments (Kakde et al., 2012).

It is also observed that the fungal association with the tomato fruit is also varied from market to market. Maximum fungi were recovered from the fruit samples collected from Dadar market (15 species) followed by Ghatkopar (11 species), Fort (10 species) and Andheri (6 species). Total number of fungal species associated with fruit samples in different markets of Mumbai have been depicted in the Fig #2.

Therefore, treatments and handling methods for the control of diseases are required to prevent losses. Consumer's increasing desire for high quality and nutritional foods has created a need for longer market keeping-period for both domestic and export markets. This is especially true for tomatoes which ranks number one among vegetables, contributing vitamins and minerals availability to consumers (Rick, 1978).

Table: 1: Frequency of occurrence of Fungal Isolates from Different locations

Location	Fungal species	Percentage frequency (%)
Fort	*Alternaria altarnata*	44
	Alternaria solani	18
	Aspergillus flavus	70
	Aspergillus niger	98
	Cladosporium sp.	8
	Colletotrichum gloeosporioides	25
	Fusarium oxyspsporam.	53
	Geotrichum candidum	5
	Penicillium digitatum	15
	Rhizopus stolonifer	18
Ghatkopar	*Alternaria alternata*	62
	Alternaria solani	11
	Aspergillus flavus	53
	Aspergillus niger	94
	Colletotrichum gloeosporioides	35
	Fusarium oxysporum	41
	Fusarium solani	3
	Geotrichum sp.	3
	Phoma sp.	2
	Rhizoctonia solani	6

	Rhizopus stolonifer	23
Andheri	*Alternaria alternata*	51
	Aspergillus flavus	72
	Aspergillus niger	95
	Colletotrichum gloeosporioides	34
	Fusarium oxyspsporam	58
	Penicillium chrysogenum	5
	Rhizopus stolonifer	18
Dadar	*Alternaria alternata*	53
	Alternaria solani	11
	Aspergillus flavus	73
	Aspergillus niger	95
	Botrytis cinerea	4
	Cladosporium sp.	14
	Colletotrichum gloeosporioides	44
	Fusarium oxysporum	45
	Fusarium sp.	14
	Geotrichum sp.	9
	Penicillium chrysogenum	39
	Phoma sp.	5
	Rhizoctonia solani	9
	Rhizopus stolonifer	20
	Verticillium sp.	3

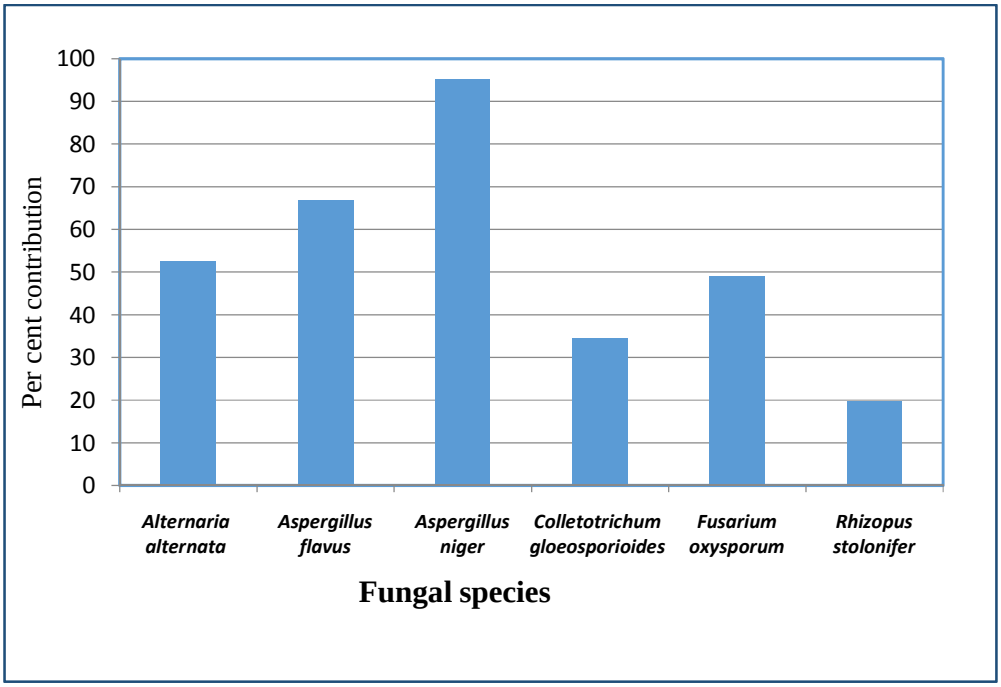

Fig.1: Per cent contribution of major fungal species associated with tomato fruit in different markets of Mumbai

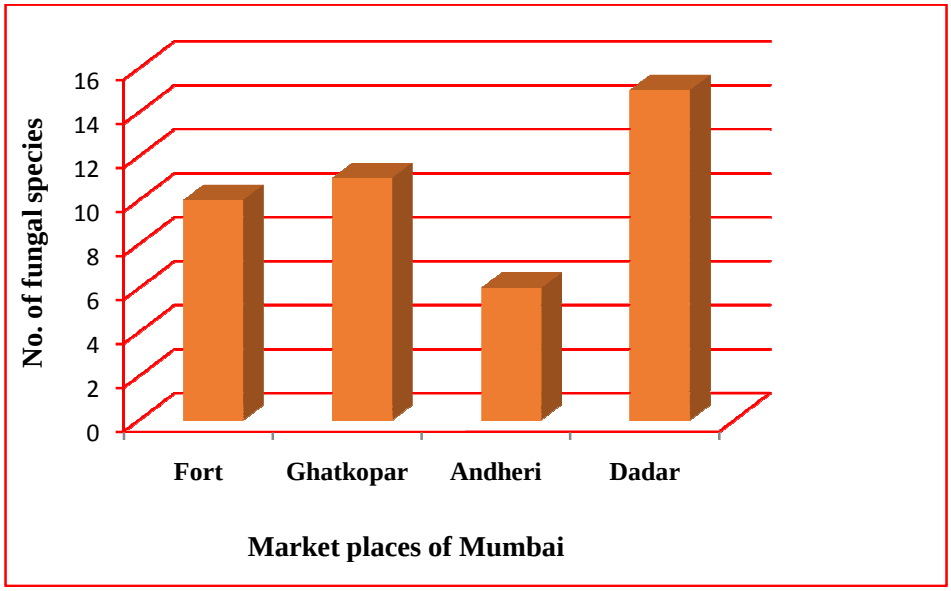

Fig.2: Total number of fungal species associated with fruit samples in different markets of Mumbai

Conclusion:

In the present investigation many post harvest fungal diseases were observed. The major fungi associated with post harvest disease were species of *Aspergillus, Penicillium, Fusarium, Alternaria* etc. Of these diseases some are came from the field itself but major fungi came from the local atmosphere. The climate of Mumbai is hot and humid. The spoilage of tomatoes due to fungi was observed more because such condition is favorable for many fungi. Many fungi reported in this investigation are not reported as regular pathogen (*Aspergillus, Rhizopus, Penicillium* etc.) They are also called as opportunistic fungi and grow on the rotten vegetables and thrown away materials. If they get organic substrate like thrown away materials they grow profusely and produce the spores enormously. The spore load of such opportunistic fungi will be increased and settles on the fresh salable articles, associated with them and responsible for the post harvest losses.

The present study revealed that tomatoes which are marketed in different markets of Mumbai are contaminated by several fungal pathogens and opportunistic fungi. Tomato fruits were more prone to infection by fungal pathogens. Many fungal isolates such as species of *Fusarium, Aspergillus, Alternaria,* and *Penicillium* appeared to be most active of all the pathogens responsible for the degradation and deterioration or losses of economic resources as well as production of mycotoxins. Therefore it is recommended that more research be focused on the methods of prevention and control of spoilage of tomatoes by fungal isolates.

There is probably a cyclic relation between the prevalence of fungal spores in the air and post harvest diseases or decay of the vegetable and fruits. That may be due to the growth of fungi on dumped plant materials, packing leaves, infected vegetables and fruits, where fungi grow saprophytically and produce spores enormously which is

responsible for the contamination of the environment. Hence, the salable articles like vegetable and fruits should sell in good conditions where plant debris and thrown away materials, packaging materials should not be available to grow the opportunistic fungi ion the market environments.

References:

- Abdel-Mallek, A.Y., Hemida, S.K. and Bagy, M.M.K. (1995). Studies associated with tomato fruit and effectiveness of some commercial fungicides against three pathogens. Mycopathological 130: 109-116.
- Ainsworth, G. C., Sparrow, F.K., Sussman, A.S. (1972).The Fungi, 4A. Academic Press, New York.
- Barnett, H.I. (1987). Vegetables and Related Products. In: Beuchat. L.R (Ed), Food and Beverage Mycology, 129-154.Van Nostrandreinhold, New York.
- Barnett, H.I. (1960). Illustrated Genera Of Imperfect Fungi. Burgess Publishing Co. Min-Neapolis.
- Bullerman, L.B. (1979). Significance Of Mycotoxins to Food Safety And Human Health.
- Di Mascio P, Kaiser S, Sies H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989; 274:532-538
- Douglas, B. (1922). A new Alternaria spot of tomatoes in California. Phytopathology, 12: 146-148.
- Eckert, J.W. & Sommer, N.F. (1967). Control of diseases of fruits and vegetables by postharvest treatment. Ann. Rev. Pl. Pathol. 5: 391-432.
- Ellis,M.B. (1971). Dematiaceous hyphomycetes. Publisher Common wealth Agricultural Bureaux. Common wealth Mycological Institute. Kew, Surrey (Uk).
- Frazier, W.C. (1967). Food Microbiology. Mcgraw-Hill Book Co.,Inc,New York.
- Gilman, J.C. (1957). A Manual Of Soil Fugi.Iowa State Uni.Press.Ames.
- Ingold, C.T. (1974): Fungus Spores:Their Liberation And Dispersal.Oxford University Press,London And New York.
- Jerry A. Bartz, Steven A. Sargent, and Michael Mahovic (2017). Guide to Identifying and Controlling Postharvest Tomato Diseases in Florida. IFAS extension, University of Florida, HS866, U.S. Department of Agriculture, UF/IFAS Extension Service, University of Florida, IFAS, Florida A & M University Cooperative Extension Program, and Boards of County Commissioners Cooperating. Nick T. Place, dean for UF/IFAS Extension.:1-17.
- Kakde, U.B. (2017). Mycotoxins and its impact on human populations. MOJ Bioequiv Availab. 2017;3(5):131-132.

- Kakde, Umesh B., Kakde, Hemalata U., Saoji, Aarti A. (2001). Seasonal Variation of Fungal Propagules in a Fruit Market Environment, Nagpur, India. Aerobiologia 17: 177-182.
- Kakde, Umesh B. and Kakde, Hemalata U. (2012). Incidence of Post-Harvest Disease and Airborne Fungal Spores in a Vegetable Market. An International Journal Acta Bot. Croat. 71 (1), 147-57
- Kutama, A.S., Aliyu, B.S., Muhammed, I. (2007). Fungal pathogens associated with tomato wicker storage baskets. Science world journal, 2:345-378
- Lund, B.M. (1992). Ecosystems in vegetable foods. J. Appl. Bact., 73(Suppl 21): 115S-135S.
- Mujib, U. R., Naushad, K. and Inayatullah, J. (2007). Post-harvest losses of tomato crop. Sarhad Journal of Agriculture, 23(4): 1279
- Ogaraku, A.O., Alanana, J.A. and Omananyi, P.O. (2010). Decay of tomato (Lycopersium esculentum Mill) and vitamin C content of infected fruits. In : Keffi, Nasarawa State. PAT 6(2): 91-98.
- Osakwe, J.A., Chuku, E.C. and Daddy-West, C. (2010). Influence of growth media on tomato (Lycopersicum esculentum Mill) Molds. Journal of Scientia Africana 9(2): 93-101.
- Raper, K.B., Fennell, D.I. (1965). The Genus Aspergillus.The Williams And Wilkins Co. Baltimore
- Ratnam, C.V. and Nema, K.G. (1967). Studies on market diseases of fruits and vegetables. Andhra Agricultural Journal 14: 60-65.
- Rick, C.M. (1978). The tomato. Scientia Americana 239(2): 76.
- Sharma, R.R., Singh, D. and Singh, R. (2009). Biological Control of Postharvest Diseases of Fruits and Vegetables by Microbial Antagonists: A Review, Biological Control, 50: 205-221.
- Smith, G. (1969): An Introduction to Industrial Mycology. Edward Arnold Ltd, London.
- Sokhi, S.S., Sohi, H.S. (1982). Assessment of losses in tomato caused by buckeye rot. Indian Phytopathol., 35: 675-676.
- Srivastava, M.P. and Tandon, R.N. (1966). Post-harvest diseases of tomato in India. Mycopathologia et Mycologia Applicata, 29 (3-4): 254.-264
- Talvas, J., Caris-Veyrat, C., Guy, L. (2010). Differential effects of lycopene consumed in tomato paste and lycopene in the form of a purified extract on target genes of cancer prostatic cells. Am J Clin Nutr. 2010 Jun; 91(6):1716-24.
- Willie, T.D., Morehouse, L.G. (1978). Mycotoxic Fungi, Mycotoxins, and mycotoxicosis. Marealdekkarinc., New York.