A Study of Fungal Diseases Occurring on Stored Tomatoes of Balkhu Agriculture and Vegetable Market, Nepal

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ABSTRACT

Tomatoes are one of the most widely produced and consumed vegetable in Nepal. Fungal pathogens deteriorate the quality and quantity of tomato and cause health hazards to the consumers as well as economic loss to the traders. This study was carried out to identify some fungal diseases associated with post-harvest deterioration of stored tomato fruits in Balkhu Agriculture and vegetable Market of Kathmandu, Nepal. Collected samples were cultured in Potato Dextrose Agar (PDA) media in complete randomized design. Fifteen species of fungi namely *Alternaria alternata, A. solani, Aspergillus niger, Botrytis cinerea, Fulvum fulva, Colletotrichum truncatum, Curvularia spicifera, Fusarium oxysporum, Dipodascus geotrichum, Mucor mucedo, Penicillium chrysogenum, Phytophthora infestans, Boeremia exigua, Pythium aphanidermatum* and *Rhizopus stolonifer* were identified. These were responsible for 14 different diseases of Alternaria fruit rot, Anthracnose, Black mold rot, Botrytis Bunch Rot, Damping off/ fruit rot, Drechslera mold, Fusarium rot, Mucor rot, Penicillum rot, Boeremia blight, Phytophthora rot, Rhizopus rot, Russet, and, Sour rot. The presence of these fungi and corresponding rot diseases on stored tomato indicates the need for management of fungi, farm sanitation and improved market in order to prevent field-to-storage transmission of pathogen.

Keywords: Consumers, health hazards, pathogen, post-harvest, traders

INTRODUCTION

Tomatoes (*Lycopersicum esculentum* L), one of the most popular and indivisible ingredients of human diet, are very popular use for vegetable and also in fruit. They are highly produced Solanaceous vegetable crop only after potato all over the world. Approximately 182.3 million tons of tomatoes are produced on 4.85 million ha. each year in the world (FAOSTAT, 2019).

Tomatoes are non-starchy and most significant source of dietary lycopene and ascorbic acid containing antioxidants, lycopene, ascorbic acid and phenols (George *et al.,* 2004) together with vitamin-carbohydrates, proteins, fats and potassium. (Talvas *et al.,* 2010). Lycopene is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and its products. Lycopene being efficient quencher of singlet oxygen and free radicals provides protection against a broad range of epithelial cancers (Mascio *et al.,* 1989). The consumption of tomato also reduces the risk of cardiovascular disease, osteoporosis, and ultraviolet light-induced skin damage (e.g. sun burn) and cognitive dysfunction.

The crops were originated in western South America and Central America (Wamach, 2005) thereafter widely adapted to variable climatic conditions but still they are vulnerable to varieties of fungal diseases during production, harvesting, transportation and storage. In storage condition, they are often infected by several species of fungi such as *Alternaria alternata, Collectrotrichum truncatum, Phytophthora infestans, Pythium aphanidermatum, Dipodascus geotrichum, Fusarium oxysporum, Curvularia spicifera, Cladosporium* sp.*, Penicillum chrysogenum, Mucor mucedo, Botrytis cinerea,* etc., causing different diseases with distinct symptoms. They hinder the production of tomatoes leading to the severe economic loss. Moreover, they can lead to serious human health problem if consumed. Among them, tomato infected by *Fusarium* sp. is more dangerous to human health because they produce mycotoxins (Jofee, 1986; Nelson *et al*., 1990). Further, Alternaria is main decay causing organism of postharvest tomato fruit (Agrawal *et al*., 1950) and Alternaria rot has been considered most prevalent disease and causes huge losses to tomato thus making tomatoes unfit for consumption (Douglas, 1922). *Alternaria solani, Rhizopus stolonifer*, and *Aspergillus niger* are the most common pathogen and cause loss of 52.7%, 35.9% and 25% respectively in tomato fruit in Egypt (Mallek *et. al*., 1995). Fusarium rot caused by *Fusarium oxysporum* is reported as the most destructive on ripened tomato in the U.S. (Benyal *et al.,* 2008). They can produce mycotoxin that is carcinogenic. Phytophthora rot is caused by *Phytophthora infestans* (Mills, 1940). These postharvest losses are more severe in developing than in developed countries (Enyiukwu *et al.,* 2014). The magnitude of postharvest loses always vary from one country to another and one season to another and even one day to another (Mujib *et al.,* 2007) so it is a challenging task for controlling these disease on tomatoes for researchers. Therefore, the aim of this study was to investigate the occurrence of fungi associated with tomatoes in stored conditions in agricultural and vegetable market of Balkhu (BAVM), Kathmandu, Nepal.

Study area

Balkhu Agriculture and Vegetable Market is one of the busiest trade and transit area located at southern side of Kathmandu valley and lies at an altitude of 1311 masl. It has a pleasant climate with average summer temperature ranging from 25^{o}C to 35^{o}C and 2^{o}C to12^{o}C in winter. The average annual temperature is 18.1 ^{o}C. About 1505 mm of precipitation falls annually (https://en.climate- data.org/). Tomatoes are brought to BAVM by the local traders from different places of Nepal. Among them, tomatoes brought from three places Bara district (Simraumgadh), Dhading district (Naubise), and Kavre district (Panauti) and stored in BAVM were chosen for investigation. Tomatoes brought from Simraumgadh of Bara district was considered as site A. Similarly, tomatoes brought from Naubise of Dhading district was consider as site B and tomatoes brought from Panauti of Kavre district was considered as site C. The tomatoes of Panauti was brought to BAVM from secondary source- Kalimati vegetable market, which is the center vegetable market of Kathmandu valley of Nepal.

Site A = Bara (Simraumgadh) is in Terai region of Nepal.

Site B = Dhading (Naubise) is in hilly region.

Site C = Kavre (Panauti) is also in hilly region of Nepal.

MATERIALS AND METHODS

Collection of Samples

The infected tomatoes, 30 each, from three different study sites- A, B, and C brought and

stored in BAVM were collected during November, 2019 to March 2020.

Laboratory Analysis

Isolation

The collected samples were kept in sterilized paper bags and brought to the Central Department of Botany, Tribhuvan University laboratory. Each of the samples was leveled and photographed. The Transverse Section (T.S.) of the infected part of the tomato was prepared and examined under the microscope for identification. For confirmation, the pathogens were separated from its host and grown in sterile culture medium plates. Plant pathogenic fungi were isolated by

planting surface sterilized bits of the infected plants tissue on sterilized media. For surface sterilization, four petri plates were arranged in a row near flame, under sterile condition. In the first petridish 70% ethyl alcohol was filled and the rest three dishes were filled with sterilized water. Small bits of infected crops tissue were sterilized individually by placing them in ethanol for 1-2 minutes, and then transferred to sterile water in the next dish after 1-2 min. Then, they were moved to the next dish of sterile water. Thus, surface sterilized bit was aseptically transferred on the sterilized solidified Potato Dextrose Agar (PDA) medium in petri plates with the help of sterilized forceps.

Culture

The petri plates containing surface sterilized materiel on sterilized medium were sealed with paraffin tape and incubated in inverted position at 25 $^{\circ}$C for 72 hours for growth (Pathak, 1984).

Subculture

After 72 hours, the petri plates were taken out from the incubator and photographed. Then, they were brought to subculture process in which three inoculums dishes were prepared with the help of borer from 3 days old culture and transferred to three new petri plates filled with new PDA media separately. The petri plates were sealed with paraffin tapes and kept for incubation at 25^{o} C for 10 days. Thus obtained pure culture was photographed.

Identification

The fungi were carefully transferred on the cello tape and mounted on the slide containing mixture of lacto phenol and cotton blue in order to stain the material. Thus prepared slides were examined under digital microscope (Olympus microscope Model No. CX22 Japan). The photographs were taken under immersion oil. The morphological characters of the fungi were studied under high power (10x X 40x). The pathogen were identified with the help of diagnostic morphological characteristics seen under microscope as well as concerning standard literatures (ARX, 1974; Bessey, 1950; Gilman, 1957; Barnett, 1960; Ainsworth, *et al.,* 1972), expertise and web surfing on online data base (Index Fungorum, Mycobank.org).

RESULTS AND DISCUSSION

Altogether 15 species of pathogenic fungi were identified from tomatoes brought to BAVM from three sites A, B and C. and stored in the same market. The varied fungal pathogens associated with the tomato diseases in each site are tabulated in table 1. Eight species (*Alternaria alternata* (Plate 1), *Alternaria solani* (Plate 2), *Aspergillus niger* (Plate 3), *Collectrotrichum truncatum (*Plate 6), *Fusarium oxysporum* (Plate 8), *Dipodascus geotrichum* (Plate 9), *Mucor mucedo* (Plate 10) and *Rhizopus stolonifer* (Plate 15) were obtained in all three sites of A, B and C. 12 species. *Alternaria alternata* (Plate 1), *Alternaria solani* (Plate 2), *Aspergillus niger* (Plate 3), *Botrytis cinerea* (Plate 4), *Fulvum fulva* (Plate 5), *Colletotrichum truncatum* (Plate 6), *Curvularia spicifera* (Plate 7), *Fusarium oxysporum* (Plate 8)*, Dipodascus geotrichum* (Plate 9), *Mucor mucedo* (Plate 10), *Pythium aphanidermatum* (Plate 13) and *Rhizopus stolonifer* (Plate 15) were found in site A and B. Nine species *Alternaria alternata* (Plate1), *Alternaria solani* (Plate 2), *Aspergillus niger* (Plate 3), *Colletotrichum truncatum* (Plate6), *Fusarium oxysporum* (Plate 8), *Dipodascus geotrichum* (Plate 9), *Mucor* *mucedo* (Plate 10), *Penicillum chrysogenum* (Plate 11) and *Rhizopus stolonifer* (Plate 15) were common in site B and C. And eight species (*Alternaria alternata* (Plate 1), *Alternaria solani* (Plate 2), *Aspergillus niger* (Plate 3), *Colletotrichum truncatum* (Plate 6), *Fusarium oxysporum* (Plate 8), *Dipodascus geotrichum* (Plate 9), *Mucor mucedo* (Plate 10*)* and *Rhizopus stolonifer* (Plate 15) were common in site A and C. Similarly, on the basis of frequency of occurrence of the

species, eight dominant (D) species were found in all three sites A, B and C. Five moderate (M) species *Botrytis cinerea* (Plate 4), *Fulvum fulva* (Plate 5), *Curvularia spicifera* (Plate 7), *Penicillum chrysogenum* (Plate 11) and *Pythium aphanidermatum* (Plate 13) were found in two study sites and two rare (R) species *Boeremia exigua* (Plate 12) and *Phytophthora infestans* (Plate 14) found in only one of the study site B (table 1).

It is found that among all 15 species 12 species (except *Boeremia exigua*, *Penicillium chrysogenum* and *Phytophthora infestans*) were recorded from site A, whereas all 15 species were recorded from site B and 9 species (*Alternaria alternata, Alternaria solani, Aspergillus niger, Colletotrichum truncatum, Fusarium oxysporum, Dipodascus geotrichum, Mucor mucedo, Penicillum chrysogenum* and *Rhizopus stolonifer* were obtained from site C.

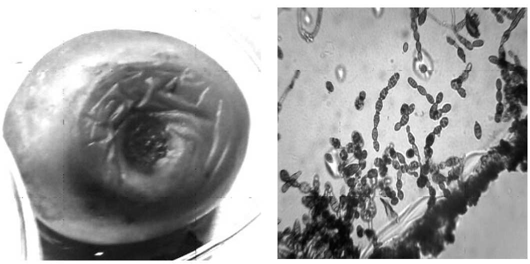
TABLE 1. Comparative occurrence of fungal pathogens and their corresponding diseases.

S. N.	Name of fungi	Site A	Site B	Site C	Category	Diseases
1.	*Alternaria alternata*	+	+	+	D*	Black mold rot
2.	*Alternaria solani*	+	+	+	D	Alternaria rot
3.	*Aspergillus niger*	+	+	+	D	Black mold rot
4.	*Botrytis cinerea*	+	+	-	M**	Grey mole rot
5.	*Cladosporium flavum*	+	+	-	M	Scab/ Cladosporium rot
6.	*Colletotrichum truncatum*	+	+	+	D	Anthracnose rot
7.	*Curvularia spicifera*	+	+	-	M	Drechslera mold rot
8.	*Fusarium oxysporum*	+	+	+	D	Fusarium rot
9.	*Dipodascus geotrichum*	+	+	+	D	Sour rot

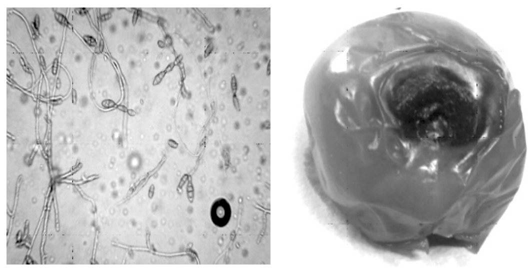
10.	*Mucor mucedo*	+	+	+	D	Mucor rot
11.	*Penicillum chrysogenum*	-	+	+	M	Penicillium rot
12.	*Boeremia exigua*	-	+	-	R***	Boeremia rot
13.	*Pythium aphanidermatum*	+	+	-	M	Pythium rot
14.	*Phytophthora infestans*	-	+	-	R	Phytophthora rot
15.	*Rhizopus stolonifer*	+	+	+	D	Rhizopus rot
Total numbers of species		12	15	9		
Numbers of species in percentage		80%	100%	60%		

*Dominant, **Moderate, ***Rare.

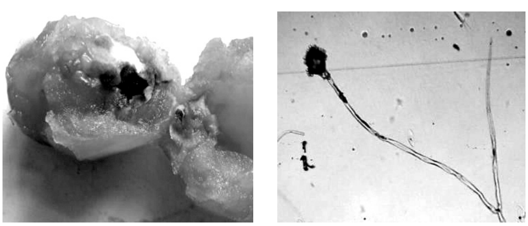
In present study, the maximum numbers (15 species) of fungi were recorded from site B (100%) then site A, 12 species (80%). Site C, had least in number i.e. 9 species (60%) as depicted in table 1.



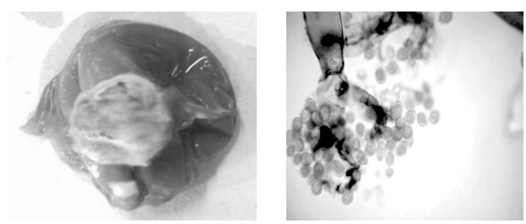
1. Alternaria alternata



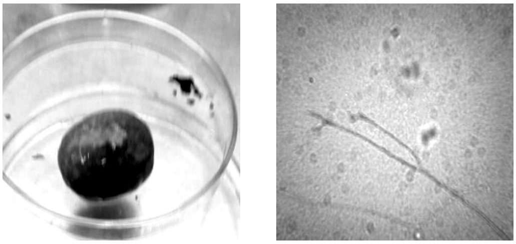
2. Alternaria solani



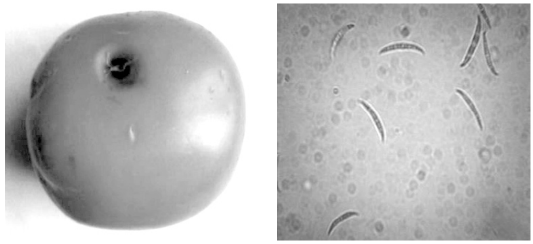
3. Aspergillus niger



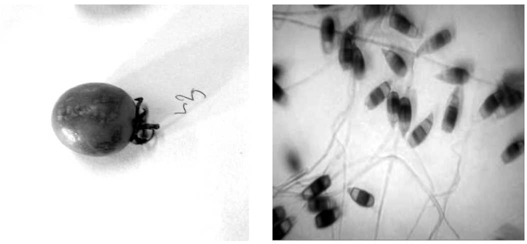
4. Botrytis cinera



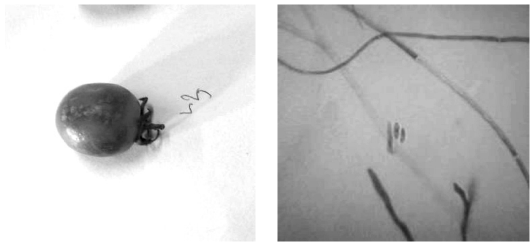
5.Cladosporium sp



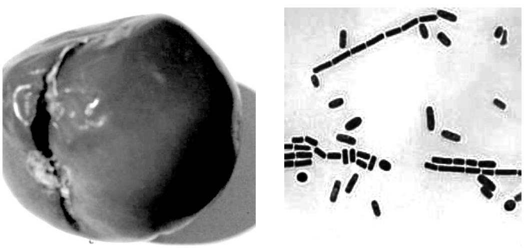
6. Colletotrichum truncatum



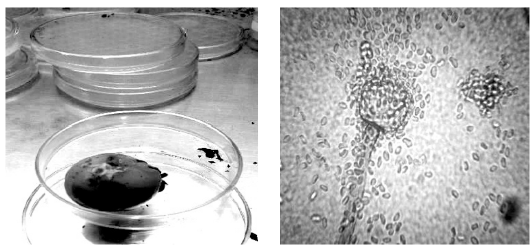
7. Curvularia spicifera



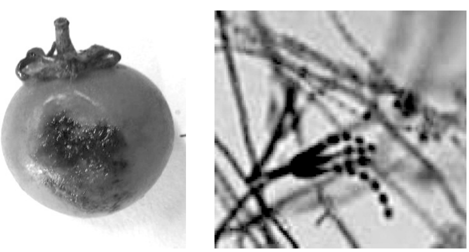
8. Fusarium oxysporum



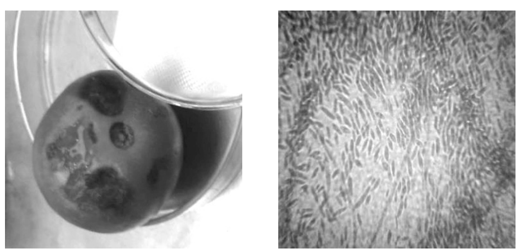
9. Dipodascus geotrichum



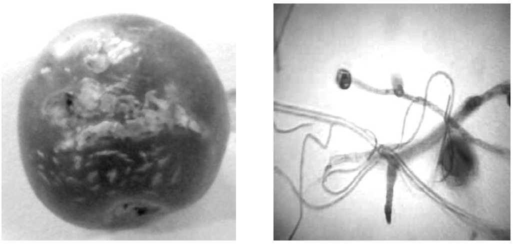
10. Mucor meucedo



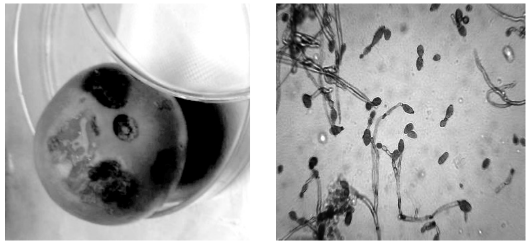
11.Penicillum chrysogenum



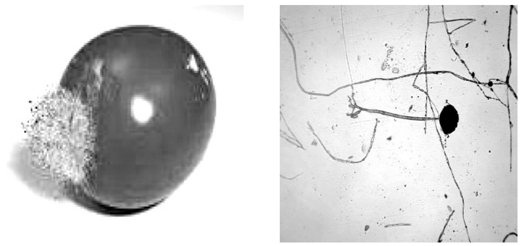
12. Boeremia exigua



13.Pythium aphanidermatum



14.Phytophthora infestan



15. Rhizopus stolonifer

PHOTO PLATES 1-15. Disease symptoms and corresponding pathogens obtained during the investigation.

Occurrence of pathogens on tomatoes basically depends upon the sources of tomato and fungal bio-aerosols of market environment where they are exposed (Kakde & Kakde, 2012). Temperature, moisture and bioaerosol are the major factor for producing infection on tomatoes. The stored tomatoes became highly susceptible due to wound occur (Sajad *et al.,* 2017). In present study, availability of 15 pathogenic fungal species was varied in different study sites (table 1). Same number of species was reported by Manandher *et al.* (2017). They reported 15 species of fungi from 342 tomato crop samples along with other solanaceous crops collected from different part of the country. They reported *Alternaria alternata*, *Alternaria solani* and *Phytophthora infestans* similar to this investigation. They also reported *Cladosporium* sp., *Collectrotrichum* sp., *Fusarium* sp., *Phoma* sp. and *Pythium* sp. that cause fruit rot and other diseases on crop plants.

Of the 15 reported species, eight were most common and dominant (table 1). Similar results was reported from Mumbai, India by Rodrigues & Kakde (2019). They mentioned that *A. niger,* *A. flavus, A. alternata, Collectrotrichum* sp*., Rhizopus* sp. and *F. oxysporum* were the most common and frequently isolated. While *Botrytis cinerea, Penicillum digitatum, P. chrysogenum, Boeremia* sp*., Cladosporium* sp. were the least fungi they isolated during investigation which was almost similar to this investigation. And similar report was given in Nagpur, India by Kakde & Kakde (2012).They reported that the fungi like *Aspergillus, Penicillum, Cladosporium, Fusarium* and *Alternaria* were the most frequent associated fungi isolated from vegetable and fruits. Similarly, Shrestha (2005) had included four tomato pathogens e.g. *Alternaria* sp., *Colletotrichum* sp., *Stemphyllum* sp. and *Verticillum* sp. These species were the most prevalent in the commercial market and also found to be responsible for most of the decay of vegetables and fruits during storage.

All of the species reported in present investigation are responsible for 14 different corresponding diseases (table 1). Most of them are respective rot disease. Wani (2011) reported nine fungal rot diseases on postharvest tomato, among them seven were similar reported in present investigation that are Alternaria rot, Anthracnose rot, Mucor rot, Blue mole rot, Phytophthora rot, Phomopsis blight, Fusarium rot caused by respective pathogen of *Alternaria solani, Colletotrichum truncatum, Mucor Mucedo, Penicillum* sp., *Phytophthora infestans, Boeremia destructive* and *Fusarium oxysporum* respectively. Similar to this investigation, Massoud (2013) also isolated *Aspergillus, Acremonium, Alternaria, Fusarium,* and *Penicillum* in *Lycopersicum esculentum* from Aswan, Egypt. Chigoziri, *et al.* (2018) also reported *Aspergillus flavus, Colletotrichum capcisi,* and *Pythium* sp. from Nigeria.

Bartz *et al*. (2017) described 11 fungal post-harvest diseases along with bacterial postharvest diseases found in Florida. Among them, nine of the fungal rot diseases namely Fusarium, Phoma, Anthracnose, Cladosporium, Grey mole, Phytophthora, Rhizopus, Black mold and Sour rot, similar to results obtained in present investigation.

Thus, during this investigation, 11 fungal rot diseases of Alternaria, Anthracnose, Black mold, Fusarium, Mucor, Penicillium (Blue mold rot), Phytophthora, Pythium, Phomopsis blight, Rhizopus and sour rot were described more or less by different researchers from Nepal and abroad**.** Kohl *et al.* (2015) only reported scab epidemic in Dabrowice on Cortland Apple.

The tomatoes in Balkhu market were imported from Dhadhing, Bara, Kalimati as well as from different places of the country. It is recommended to grow Srijana variety in the context of Bara district (Gurung *et al.,* 2020). Local farmers have been supplying vegetables produced in Dhading to market in Balkhu of Kathmandu (Shrestha, 2017). Some similar pathogenic fungi were seen in the dominant species in all three study sites, as they were transmitted from fields with suitable temperature for their growth. Some dissimilarity was also observed that must be due to verities of tomatoes and market environment where opportunist fungi grow. Both biological and physiological damage during the harvest and transportation phase coupled with large amount of water and soft endocarp makes tomato more susceptible to spoilage by fungi (Asan & Ekmeki, 2002; Sajad *et al.,* 2017). The tomatoes brought from Dhading district were local tomatoes having thin epidermis with soft endocarp which made it easier for the fungus to infect the tomatoes may be the reason for more infection found in tomatoes from Dhading district.

Till now, many researches on tomato diseases have been carried out by NARC in order to produce resistant varieties. Most of them were concentrated on late blight. For a long time, NARC has researched to produce late blight resistant tomato caused by *Phytophthora infestans*. NARC has been involved in hybrid breeding of tomato with special focus on developing resistant FIs on BW and LB Srijana. Some of the new hybrids are promising in this year and among them to superior tomato hybrid lines; HRA 14x7 and HRA20XHRD2 have been submitted to national seed board for releasing (NARC, 2020). In co-ordination varietal evaluation at Khumaltar, cultivar STMO2 was least affected by late blight disease whereas CLN2545B, HRD109, STM02 and Pusa ruby were least affected by Septoria disease (NARC, 2019). However, investigations on stored tomato diseases caused by other pathogenic fungi haven't been carried out so far. Thus, it is the first investigation depicting occurrence of fungal pathogens and associated diseases on post-harvest tomatoes in Nepal.

Most of the fungi obtained in this investigation were soil fungi that are transmitted from field or during transportation and storage. Local tomatoes are more prone to infection by fungi present in bio-aerosol of market environment. Directly or indirectly fungi infected fruits and vegetables sold by traders cause ill consequences on the consumer's health. Therefore, control measures shoul be implied by farmers and traders. Late blight disease can be managed by rational use of bio-pesticides and fungicides, krilaxyl and dithane M-45 (Shrestha & Ashley, 2007).

Disease of fruits rots are generally caused by opportunistic pathogen that normally lives on plant debris. However, these opportunists can infect tissue that are wounded or exposed or diseased (Bertz *et al.,* 2017). Thus fruit rots can be minimized in field by the employment of strict sanitation measures along with careful handling during transportation and storage time. Resistant variety should be used by farmers. Traders should use strict sanitation measures in shops. They should keep out bruised, rotten and infected fruits separate and dispose them accordingly so that the fresh fruits won't get infected.

Since human pathogen do not visibly affect the fruit their presence may be unknown at the time of packaging and marketing (Bertz *et al.* 2017) which necessitates consumer's knowledge about the symptoms of diseases in order to identify the diseased tomatoes. In addition, well- managed, sanitized, well-facilitated vegetable market should be provided by local government as services for local people. Therefore, this study provides the necessary awareness of fungal disease in public. It may also help to minimize losses caused by postharvest fungal diseases to local traders and will be helpful to build up concrete strategy for management of postharvest fungal disease of tomato. As the study of diseases on tomatoes is important and concerned with public health, it is very necessary to carry out further research works on tomato diseases in future.

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