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Studies on post-harvest diseases of Tomato

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ABSTRACT

The survey carried out revealed that factors such as poor grading, packing containers, means of transport, duration between harvest and transport to the market, pests and diseases have significant impact on post-harvest losses. Tomato fruits were usually spread on the ground waiting for grading after harvest. Mixing of healthy and infected tomato fruits during harvesting possibly increased chances of the spread of disease-causing micro-organisms to healthy fruits. The major causative agents of post-harvest spoilage of tomatoes are bacteria and fungi. The isolated pathogens were *Rhizopus* spp., *Fusarium* spp., *Geotrichum* spp., *Botrytis* spp., *Curvularia* spp., *Bipolaris* spp. The results of pathogenicity test from this study revealed that all tomato fruits showed symptoms of rot while the un-inoculated control fruits showed no symptoms of rot. However, the rate of rot varied significantly between the pathogens with *Rhizopus* spp. being the most virulent pathogen causing the most damage (100 % rot) within two days. *Bipolaris* spp. caused the least damage meaning that it was not one of the most damaging pathogens.

Keywords: Tomato, Post-harvest diseases, Rot, Virulent

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae and it is an annual sub-tropical fruit vegetable crop. The crop originated from South America and was introduced to Europe in the 16th Century and later to East Africa by colonial settlers in early 1900 (Wamache, 2005). Tomato plays a vital role in meeting domestic and nutritional food requirements, etc. (Sigei *et al*., 2014). The crop is grown for both fresh domestic and export market but there is increasing demand for processed tomato products (Mungai *et al*., 2000).

Tomato production is constrained by factors such as poor pre-harvest practices, adoption of poor production techniques, rough handling and moisture condensation causing pathogen infestation (Kader, 1992). Packaging in bulk without sorting and grading of produce, damage

during transport and storage due to mechanical injuries are other factors contributing to post-harvest losses (Kader, 1992). Inadequate storage, distance and time-consuming market distribution, poor access to the market, post-harvest spoilage micro-organisms and cultivars disposition to diseases causes high post-harvest losses of tomatoes (Kader, 1992).

It has been estimated that 20-50 % of tomato fruits harvested for human consumption are lost through microbial spoilage while other losses result from damage by dynamic stresses during transit, and through rough handling during loading and unloading (Kader, 1992; Okezie, 1998). Post-harvest decay remains a major challenge in tomato production.

During the survey of local markets it was recognized that among fruits and vegetables tomato is highly perishable and has very short shelf life and easily get spoiled by many fungi hence tomato has been selected as fruit and vegetable commodity for study.

The main objectives of the study are-

- Survey of local markets for the collection of diseased tomato fruits.
- To assess and document the causes of post-harvest losses of tomato
- Isolation, identification, purification and maintenance of fungal isolates.

MATERIAL AND METHOD

The experiments were carried out at the Laboratory of Department of Botany, B. Raghunath Arts, Commerce & Science College, Parbhani. The experimental techniques, procedures and the formulas were adopted during the research work are discussed below:

1. Study Area

The study area *i.e.* Parbhani is located at 19.27°N 76.78°E. It has an average elevation of 347 meters. In the northeast of district on the boundary of Hingoli district and Parbhani district there is extension of Ajanta ranges called Nirmal Hills. The main river in the district is Godavari river, other rivers are Purna and Dudhana which are tributaries of Godavari. Major dams around Parbhani city in Parbhani district are Yeldari dam which is on Purna river, Lower Dudhana dam is on Dudhana river, Mudgal barrage on Godavari river, Masoli Dam medium project on Masoli river, Karpara Dam on Karpara river.

2. Survey and collection of diseased material

Post-harvest diseases of tomato (*Lycopersicon esculentum* Mill.) were surveyed in different market spots at Parbhani city. The infected tomato fruits were collected from different markets of Parbhani city viz. Shanivar Bazar (SB), Kranti Chowk (KC) market and Kali Kaman (KK) market and brought to laboratory in Polythene bags (1 Kg) for further studies.

3. Percent Disease incidence

In all the shops, samples were stored in cartons, each containing 10 kg of fruit and vegetable sample. Cartons were placed in racks. For collecting data, the samples were spread over the floor and the diseased samples from each carton were sorted out. Disease incidences were calculated using the following formula.

$$\% \text{ infection} = \frac{\text{No. of infected fruits}}{\text{Total No. of fruits}} X100$$

The fruit showing typical symptoms of disease were brought to the laboratory for isolation, identification and further studies.

4. Isolation, purification and identification of pathogens of tomato fruit

Sample of rotted and diseased fruits collected from markets were used for the isolation of pathogens. The fruits showing the initial and distinct characteristic symptoms were selected for isolation of pathogen by using the method described by K.R. Aneja in his book (Experiments in Microbiology, Plant pathology and Biotechnology). The selected fruits were washed with running water in order to remove the dust particles. The fruits and vegetables were dipped in 3 per cent solution of sodium hypochlorite for 30 seconds and washed thoroughly three to four times with sterilized water to remove the traces of sodium hypochlorite, after that the infected fruit was wiped with a cotton swab dipped in 70 per cent ethanol followed by lightly flaming the tissue.

Three or four pieces of infected portion were placed on per Petri plate which was already poured with Potato dextrose agar medium (PDA: Potato-200.0g, dextrose-20.0g, streptomycin-0.3g and agar-20.0g per liter). Petri plates were incubated at 25°±2C, in an inverted position, for 5-7 days in incubator.

As soon as mycelial growth and spores of fungus become visible, bits of agar containing mycelia from the edge of developing colonies were transferred to slants for identification and further use (Aneja, 2009).

The purified pathogen was identified up to genus level based on cultural character and type of conidia produced. The culture was purified using hyphal-tip method and maintained on PDA slants in the refrigerator for further studies (Brown, 1934).

For microscopic observations, different fungal isolates were stained with lactophenol cotton blue and observed under the microscope at different magnifications.

Fungi were identified on the basis of their morphological and cultural characteristics with the help of available literature (Gilman, 1967; Ellis, 1976). The cultural characters like type and colour of growth on Potato dextrose agar medium as well as pathogenic behavior towards the host were recorded.
Morphological characters taken into account were as:
Colony: Color and growth
Mycelium: Color, branching pattern, Septation and width
Colony: Color and growth
Conidiophores: Color, size, and septation

5. Pathogenicity test

Pathogenicity test was proved by Koch's postulate. After isolation of pathogen cultures were purified and maintained. Healthy tomatoes, of the same size were washed after being surface sterilized with disinfectant rinsed with sterile distilled water. By using alcohol dipped and flamed cork-boarer, fruits were punctured with single wound centrally near stem end. Fruits were inoculated with a mycelial disc cut from the margin of growing pathogen culture. Test samples were incubated at 25±2ºC for 7 days. Re-isolation of the pathogen was carried out.

RESULTS AND CONCLUSION

The investigations on post-harvest diseases of tomato were carried out during September 2018 to January 2019 and the results thus obtained are presented below:

1. Survey for incidence of postharvest diseases tomato and collection of diseased specimen

A comprehensive survey was conducted to assess the losses of tomato caused by postharvest pathogens. In Parbhani city, the selected three locations *viz*. Shanivar Bazar (SB), Kranti Chowk (KC) market and Kali Kaman (KK) market were surveyed every month. In all, samples collected from the three markets each containing 1 kg of fruit sample.

2. Percent incidence of post-harvest disease

For collecting data, the samples were spread over the floor and the diseased samples from each bag were sorted out and the percent disease incidences were calculated by using following formula-

From the results presented in table 1, it was found that tomatoes from the markets of Shanivar Bazar and Kranti chowk market were found severely infected with different fungal species.

Table 1: Per cent disease incidence in in different market places in Parbhani city

Sr. No.	Name of Market	Per cent disease incidence
1	Shanivar Bazar (SB)	50 %
2	Kranti Chowk market (KC)	41.66 %
3	Kali Kaman market (KK)	8.33 %

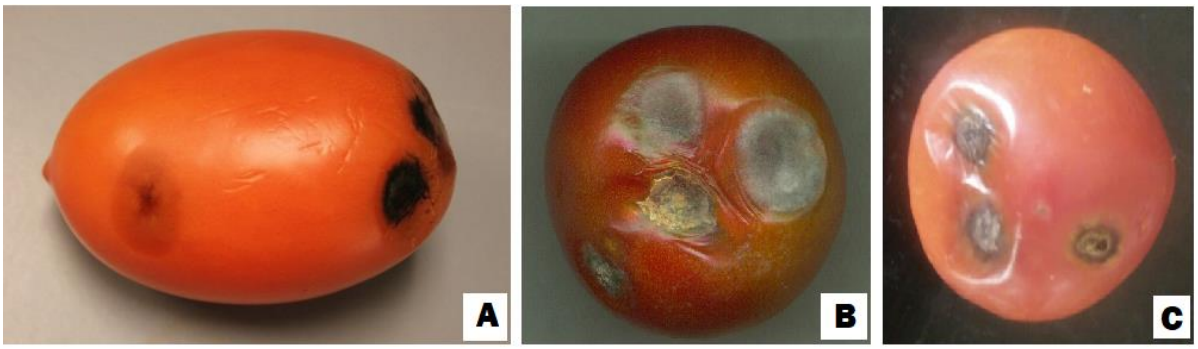

Figure : 1 Infected Tomato fruits A: *Alternaria* infection **B**: *Curvularia infection* ***C****: Rhizopus* infection

Table 2: Microscopic characteristics used for the identification of post-harvest diseases.

Sr. No.	Name of Pathogen	Microscopic characters/Identification marks
1	*Fusarium* spp.	Colonies were fast growing, mycelia extensive, cottony in culture, and pink, yellow and white in color. Conidiophores were variable, slender and simple, or stout, short, branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia; conidia (phialospores) were hyaline, variable, often held in small heads. Macroconidia hyaline, several celled slightly curved or bent at the pointed ends, typically canoe-shaped. Microconidia were also hyaline, pyriform, fusiform to ovoid, straight or curved, 1-celled, borne singly or in chains; some conidia intermediate, 2 or 3 celled, oblong or slightly curved.
2	*Botrytis* spp.	Fungal colonies growing in PDA were woolly, dark grey with a black reverse . The colonies were also fast growing but the growth was patchy or irregular. Conidiophores were long, slender, hyaline or pigmented, branched, sometimes near the apex, the apical cells enlarged or rounded, bearing clusters of conidia on short sterigmata; conidia hyaline or ash-colored, gray in mass, 1-celled and ovoid.
3	*Curvularia* spp.	Colonies of the fungus on PDA were fast growing; brownish and cottony. The reverse was dark brown. Conidiophores were straight to flexious, multi-septate, usually simple but sometimes branched, brown and bearing spores apically. Conidia were dark, septate, end cells lighter, 3 to 5 celled, more or less fusiform, typically bent, with one of the central cells enlarged and darker.
4	*Geotrichum* spp.	The fungus colony grew in PDA being low, flat, white and leathery with no reverse pigmentation. Hyphae were hyaline septate, branched and broke up into chains of hyaline, smooth, one-celled, subglobose to cylindrical, slimy arthroconidia (ameroconidia) by the holoarthric fragmentation of undifferentiated hyphae. The arthroconidia, were quite variable in size, aerial, erect or recumbent, cylindrical, hyaline, unicellular & Barrel shaped.
5	*Bipolaris* spp.	The fungal colonies were moderately fast growing; effuse, grey to blackish brown, suede like to floccose with a blackish brown reverse on PDA. Hyphae were septate and branched. Conidiophores were brown, simple, producing conidia through apical pore, resuming growth sympodially and forming conidia on successive new tips. Conidia (porospores) were brown; several celled (phragmosporous), fusoid, and straight or curved, germinating by one germ tube at each end. The spores were unique in that they were not made up of normal cells separated by septae instead the cells of spores were compartmentalized by distosepta, meaning they were contained in sacs that had a wall distinct from the outer wall of the conidium.
6	*Rhizopus* spp.	It grew rampantly filling the petridish with sparse white mycelia within four days. Colony whitish becoming grayish-brown due to yellowish brownish sporangiophores and brown black sporangia, with extensive mycelia growth in culture as it ages. The texture was typically cotton candy like. The mycelia was non septate. Sporangiophores were large with striate walls and irregular in shape. Their color ranged from almost colorless to dark brown with slightly rough-walled stolons opposite the branched rhizoids. Sporangia were globose to subglobose and blackish-brown at maturity. Columella projected into the sporangium. Sporangiospores (asexual spores) were irregular in shape and were formed within pinhead like sporangium, which break to release the spores when mature
7	*Alternaria spp.*	The mycelium is profusely branched, brownish and septate. Conidiophores: Developed singly or in small groups, branched or unbranched. Conidia: In long chains (often branched), oval to ellipsoidal, with 2-7 transverse and 1-4 longitudinal or oblique septae, tapering end to form a short beak at the apex. The number of conidia in a chain varied from 2-8.

3. Isolation and identification of pathogens

The fruit showing typical symptoms of disease were brought to the laboratory for isolation, identification and further studies.

The pathogens that were isolated and identified were *Fusarium* spp., *Botrytis* spp., *Curvularia* spp., *Geotrichum* spp., *Bipolaris* spp., *Rhizopus* spp. Among the fungi *Fusarium* spp. was the most prevalent with three species constituting 30 %. *Rhizopus* spp. constituted 21 %, *Curvularia* spp., 8 % while *Geotrichum* spp. formed 18 % of the total population. *Bipolaris* spp. constituted 5 %, *Botrytis* spp., formed 15 %.

The isolation and purification of fruit rot pathogens was carried out as per the method described in materials and methods. The pathogens, which were responsible for diseases on tomato observed during survey, and the symptoms they produced as well as their cultural characteristics.

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Conflicts of Interest: The authors declare no conflict of interest.

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