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Study on post-harvest diseases of *rabi* vegetables in Jorhat district of Assam, India

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ABSTRACT: A preliminary study was undertaken to screen the post-harvest diseases associated with some common *rabi* vegetables in the Jorhat districts of Assam, India. The infected vegetables were collected from a few local markets (Rajoi tiniali market, Unnayan market, Chowk Bazar sabji market) of Jorhat District of Assam. Isolation, purification, identification, and maintenance of the pathogenic isolates were carried out following standard protocol. The isolated fungal and bacterial pathogens were identified based on cultural and morphological characters. Pathogenicity tests of the isolated pathogens in different vegeatables confirmed association of *Alternaria* sp. in tomato, *Cladosporium* sp. in cauliflower, *Erwinia* sp. in capsicum, *Geotrichum* sp. in carrot, *Sclerotinia* sp. in pea and bean) and *Phomopsis* sp. in brinjal.

Keywords: Post-harvest diseases, pathogens, *rabi* vegetables

INTRODUCTION

The importance of fruits and vegetables, in improving the nutritional status, health, and economy of mankind need no emphasis. Vegetables are an important source of dietary fibers, minerals, antioxidants, and vitamins. Shifting from a non-vegetarian diet to vegetarian, global recognition of the importance of vegetables for human health has contributed to a steady upward trend in the vegetable production system. China is ranked first in the world and India is the second-largest producer of vegetables after China. In India vegetable crops produced in 2018 was a total of 184.40 million tonnes in an area of 10.26 million hectares with productivity of 17.97 million tonnes (Annon., 2018). Potato, tomato, chilli, onion, brinjal, cabbage, cauliflower, peas, okra, beans, melons, etc are some of the important vegetable crops predominantly grown in the country.

Diseases both pre-harvest and post-harvest are the major constraints in vegetable cultivation causing a considerable yield loss, however post-harvest diseases have not yet received the due attention (Bora *et al.,* 2016). Most of the developing countries in tropical regions with high temperatures, and humidity favours disease development in many crops (Eckert and Sommer, 1967; Droby, 2006). In the former case, pre-harvest infections remain quiescent until the fruit becomes senescent shortly after harvest or during prolonged storage. Conversely, the vast majority of post-harvest infections happen through wounds caused during harvest and subsequent handling. The plant products may get infected by microorganisms and cause rotting or decaying partially or totally (Snowden, 1992). Many of the important fungal pathogens that cause post-harvest diseases include *Aspergillus*, *Penicillium*, *Geotrichum*, *Botrytis*, *Fusarium*, *Alternaria*, *Colletotrichum*, *Phomopsis*, *Rhizopus* and *Mucor*. *Fusarium* spp. found to be associated with post-harvest of *Colocasia esculenta* (Bora *et al.,* 2020). Among the various bacterial post-harvest diseases of vegetables, bacterial soft rots are very important post-harvest diseases of many vegetables. Various factors like susceptibility of the cultivars to post-harvest disease, commodity type, the pos-tharvest environment like temperature, relative humidity, etc. and ripeness stage, disease control measures are taken, etc. are directly or indirectly affect post-harvest diseases. Post-harvest diseases may cause more economic losses than in fields because of added costs in harvesting transportation and storage. Agrios, 2005 and Kader, 2002 reported that post-harvest diseases destroy around 10-30% of the total produce of crops and in some perishable crops especially in developing countries, they destroy more than 30% of the crop yield. As per the study by the Central Institute for Post-Harvest Engineering & Technology, Ludhiana (published in 2010) postharvest losses of major agricultural produces including fruits and vegetables at the National level were estimated to the tune of about Rs. 44,000 crores per annum (Mahant, 2012). The government has stated that nearly 35-40% of the vegetables produced in the country are wasted. But as per the Indian Council on Agricultural Research (ICAR), the maximum loss in vegetables ranges between 12.4 to 18% during post-harvest. In this context addressing the post-harvest disease management needs urgent attention.

MATERIALS AND METHODS

Collection of diseased samples and isolation of the pathogen

Diseased vegetable samples were collected in sterile polypropylene bags from various local markets of Jorhat districts of Assam (Rajoi tiniali market, Unnayan market, Chowk Bazar sabji market) and preserved in the refrigerator at 4^{o} for further studies. For isolation, infected parts of the collected vegetables *viz*., tomato, cauliflower, capsicum, carrot, pea, bean, and brinjal were cut into small pieces of about 1 cm by using a sterile scalpel and surface sterilized in 1% sodium hypochlorite for about 1 minutes and rinsed thrice in sterile distilled water, as per the standard protocol. For isolation of fungal pathogen associated with, the cut specimen pieces were transferred to the Petri plates containing the potato dextrose agar (PDA) media, incubated at 28 ± 1°C for 72-96 hours, and observed periodically for the growth of the fungus. For isolation of the bacterial pathogen associated with the diseased vegetables, small cut specimen pieces of infected tissues were suspended in distilled water for 2-3 minutes and the suspension was streaked onto the surface of Petri plates containing the nutrient agar (NA) media, and the plates incubated at 28 ± 1°C for 48 - 72 hours (Carisse and Van DerHeyden, 2015) and observed periodically for the bacterial growth.

Purification and preservation of the pathogenic isolates

The fungal isolates were purified by the single hyphal tip method and maintained on PDA medium by periodical transfer throughout the present investigation. The bacterial isolates were purified by streaking the single colonies in the new Petri plates containing NA media and maintained on NA medium by periodical transfer throughout the present investigation.

For the maintenance of the purified fungal and bacterial isolates were grown on PDA and NA slants and incubated for 48-72 hours at 28 ± 1°C. The slants were then preserved in the refrigerator at 4°C for subsequent use. Periodic subculture of the fungal and bacterial pathogens were done in PDA/ NA slants, respectively during the period of the study.

Identification of the pathogenic isolates

For preliminary identification of the pathogenic isolates cultural and morphological examination was conducted following standard protocol. Morphological observations of the isolated pathogens (fungus and bacteria) were recorded by its observation through microscope under 10X, 40X and 100X power magnification. In case of fungal pathogen, a loopful of isolated fungal mycelium was placed on a clean glass slide, already mounted with a drop of lacto phenol and cotton blue, and observed under microscope for identification. On the other hand, the bacterial pathogens were identified as either Gram -ve or +ve based on their gram staining biochemical analysis followed by microscopic observation.

Pathogenicity tests

The pathogenicity test was proved in the respective vegetables, *viz*., tomato, cauliflower, capsicum, carrot, pea, bean, and brinjal, following the standard protocol, as described by Akhtar and Chaube, 2006 and Ahmed *et al.*, 2017. For this healthy vegetables free from wounds and any symptoms of the disease were selected. Vegetables were washed with tap water, surface-sterilized by dipping them in 1% sodium hypochlorite solution for 10 min, rinsed by dipping twice in sterile distilled water for at least 10 min, and dried at ambient air temperature for further study. Surface wounding of the vegetables were made on each vegetable using a pipette tip/ needle. Fungal mycelia from the pure cultured fungal isolates were inserted into wounds. For bacterial stains, the vegetables were inoculated with pathogenic bacterial suspension @ 1 X 10^{8}cfu/ ml. Inoculated vegetables were placed in a plastic box inclosing sterile paper towels moistened with sterile water and incubated at 25±2ºC for 7 days for further symptom development. The experiments to test the pathogenicity were set up with four replications and each experiment was repeated twice. Symptoms produced on the vegetables were recorded and re-isolation of the pathogens was carried out for the proof of Koch's postulates.

RESULTS AND DISCUSSION

Collection of diseased *rabi* vegetables

A survey was conducted and collected various *rabi* vegetables, *viz*., Tomato, Cauliflower, Capsicum, Carrot, Pea, Bean and Brinjal from some markets of Jorhat District of Assam, *viz*., Rajoi tiniali market, Unnayan market, Chowk Bazar sabji market for the present study (Table 1).

Isolation, purification, and identification of the pathogens

Isolation and purification of the post-harvest pathogens associated with the collected diseased vegetables were carried out as per the standard methods described in materials and methods. The specific symptoms produced by the diseased vegetables were recorded as well as cultural and morphological characteristics of the purified pathogenic isolates were carried out and described below.

Table 1. Collection of various diseased *rabi* vegetables from different markets of Jorhat district of Assam

Vegetable	Place of collection	GPS coordinates	Date
Tomato and Carrot	Rajoi tiniali Market, Jorhat, Assam	26°45'50.9"N 94°22'31.5"E	03/02/2020
Cauliflower	Unnayan Market, Jorhat, Assam	26°45'34.0"N 94°12'22.2"E	11/02/2020
Capsicum	Rajoi tiniali Market, Jorhat, Assam	26°45'50.9"N 94°22'31.5"E	13/02/2020
Pea	Rajoi tiniali market, Jorhat, Assam	26°45'50.9"N 94°22'31.5"E	17/02/2020
Brinjal and Bean	Chowk bazaar market, Jorhat, Assam	26°45'43.2"N 94°12'36.4"E	18/02/2020

Fungal and bacterial were maintained in potato dextrose agar (PDA) and nutrient agar (NA) medium by periodical transfer throughout the investigation for further studies.

Tomato

Diseased tomato fruits appear leathery, covered by a velvety mass of black spores and concentric rings present on the fruit surface. The infected tissue initiated decaying and decayed tissue was firm, dry, and brown to black coloured. Colonies of the isolated fungal pathogen associated with the tomato fruits, as observed in the PDA Petri plates are fast-growing, flat, cottony, and is covered by whitish, short, aerial hyphae. Under microscopic observation, mycelium was found septate, branched, light brown; conidia beaked, muriform, dark coloured, and borne singly. From these symptoms in the diseased tomato fruits and further cultural and morphological observation of the fungal pathogen under a microscope, it is preliminarily confirmed as *Alternaria* sp. The earlier reports of Barkai and Fauchs (1980) and Hassan (1996) also confirmed that *Alternaria* is one of the main causes of the deterioration of post-harvest tomato fruits.

Cauliflower

Blackish tissues surrounding the lesions may also occur enlarging to circular areas with concentric rings, as observed in the diseased cauliflower. The cultural characteristics of the purified fungal isolates associate with collected diseased cauliflower, as observed in the PDA Peti plates are dark mycelia, very dark greenish-black on the reverse side of the plates. Under the microscope, the fungus produced erect, dark, septate hyphae. Conidiophores were also darkly pigmented, septate, and showed tree-like branching. From these symptoms in the diseased cauliflower and further cultural and morphological observation of the fungal pathogen under a microscope, it is preliminarily confirmed as *Cladosporium* sp. An earlier report by Laemmlen (1986) proves the presence of *Cladosporium* in cauliflower and recoded similar characteristics.

Capsicum

The symptoms recorded in the diseased capsicum were light to dark-colored lesions on the fruit, water-soaked, and somewhat sunken. Bacterial ooze was also observed from the affected areas. Gram staining techniques were done for the isolated and purified bacteria and microscopic observation was confirmed as gram-negative, rod-shaped bacteria. Based on the symptomatology in the diseased capsicum and further gram staining and microscopic observation of the pathogenic bacteria were preliminarily confirmed as *Erwinia* sp. Similar findings were also recorded by Stommel *et al.* (1996) while working on soft rot of capsicum and identified the causal organism as *E. carotovora* subsp. *atroseptica* based on symptomology, carbohydrate utilization, and fatty acid profiling study.

Carrot

In the collected diseased carrots soft, watery, decaying of carrot roots was observed and further decayed areas were observed to be covered with dull, white spores of the pathogen. A white coloured fungal colony was observed in the PDA Petiplates. Hyphae and spores were white/ hyaline and conidia or arthrospores as produced by fragmentation of the hyphae were observed under a microscope. Based on the symptomatology in the diseased carrot and microscopic observation of the pathogen, it was preliminarily confirmed as *Geotrichum* sp. Similar findings were recorded by Horita and Hatta, 2016 and confirmed the presence of *Geotrichum candidum,* which causes sour rot of carrot**.**

Table 2. Preliminary identification of post-harvest pathogens from different *rabi* vegetables collected

Vegetable	Pathogen	Disease
Tomato	*Alternaria* sp.	Early Blight of Tomato
Cauliflower	*Cladosporium* sp.	Cladosporium Rot of Cauliflower
Capsicum	*Erwinia* sp.	Bacterial rot of Capsicum
Carrot	*Geotrichum* sp.	Sour rot of Carrot
Pea	*Sclerotinia* sp.	White mold of Pea
Bean	*Sclerotinia* sp.	White mold of Bean
Brinjal	*Phomopsis* sp.	Phomopsis fruit rot of Brinjal

Pea

A greyish-white moldy growth appears on the diseased pea pods, which at a later stage, looked soft, watery, odorless rot, characterized by a darkening of the invaded tissue and the presence of a rapidly spreading white mycelium. In PDA Petri plates, surface cottony, fluffy, and white mycelium was observed. Under the microscope, septate mycelium, the mycelium with age produced spherical black sclerotia, which resemble as the seeds of mustard was observed. Based on the symptomatology on the diseased pea pods and cultural and microscopic observation of the pathogen, it was preliminarily confirmed as *Sclerotinia* sp. Earlier findings say that a large number of fungal pathogens are associated with pea namely, *Ascochyta*, *Alternaria*, *Fusarium*, *Rhizoctania solani* and *Sclerotinia sclerotiorum* (Neergard, 1977, Richardson, 1990; Rathour and Paul, 2004).

Bean

Localized softened tissue and white mycelial tufts erupting through the cuticle were observed and recorded in the collected diseased beans. The beans were covered by abundant cottony, white mycelium, which at a later stage, developed into a soft, watery, odorless rot, characterized by a darkening of the invaded tissue and the presence of a rapidly spreading white mycelium. Under microscope, white, fluffy, and septate mycelium, the mycelium with age produced spherical black sclerotia, which resembled the seeds of mustard were observed. Spores were found to be spherical. Based on the symptomatology in the collected diseased beans and microscopic observation, the pathogen was preliminarily confirmed as *Sclerotinia* sp. Suslow and Cantwell (1998) also reported similar findings and mentioned that the most serious postharvest diseases of the bean are gray mould (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*).

Brinjal

In the diseased brinjal fruits, sunken lesions vary in size, the tan coloured ooze of fungal spore appeared on the lesion was observed. The fruit dried and becomes black at later stages. In PDA media fluffy type of mycelium was observed, colony colour recorded were greyish, irregular shape, the colony was observed to be compact and with a thick consistency. Under the microscope, conidia observed as sub-cylindrical and hyaline. Based on the symptomatology in the diseased brinjal fruits and cultural and microscopic observation, the pathogen is preliminarily confirmed as *Phomopsis* sp. Similar symptomological, cultural, and microscopic characteristics were also recorded by Jamir *et al.* 2018.

Pathogenicity test

Pathogenicity test showed severe decay of vegetables inoculated with their respective pathogens under laboratory conditions. On the infected vegetables, fungal growth on the vegetable surface was visible. These symptoms were similar to those observed under natural conditions. The vegetables inoculated with distilled water (control) remained free from infection and disease symptom development. Re-isolations confirmed Koch's postulates for pathogenicity.

During the present study, six fungal and one bacterial pathogen have been isolated and preliminarily identified. The preliminary identification of the isolates has been done as *Alternaria* sp. (tomato), *Cladosporium* sp. (cauliflower), *Erwinia* sp. (capsicum), *Geotrichum* sp. (carrot), *Sclerotinia* sp. (pea), *Sclerotinia* sp. (bean) and *Phomopsis* sp. (brinjal). Among the various pathogens isolated, *Sclerotinia* sp. were the most abundant genera (Table 2).

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