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RESEARCH **P**APER

Some studies on post-harvest pathogens of banana from Gorakhpur (U.P.)

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Present investigation was conducted during November 2014 to October 2015 in Gorakhpur district (U.P.) for storage diseases of banana. The eight fruit markets were visited for survey and sampling of banana. During survey, maximum disease incidence was found during July to October from Bargadwa fruit market while minimum disease incidence was found in Raptinagar between November to February; crown rot, Anthracnose and finger rot diseases were found to be dominant diseases. Total 12 fungi *viz.*, *Aspergillus flavus, A. niger, Alternaria* spp., *Botryodiplodia theobromae, Colletotricum musae, Fusarium equiseti*, *F. moniliforme*, *F. oxysporum, F. solani*, *Mucor circinelloides*, *Penicillum* spp. and *Rhizopus stolonifer* were isolated from diseased fruit samples. Pathogenicity test also fulfilled Koch's postulates.

Key words : Banana, Disease incidence, Post-harvest diseases, Fungal isolation

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INTRODUCTION

Banana is the most important commercial fruit crop grown in tropical and subtropical regions of India having a great socio-economic significance. It is referred as Kalpatharu (a plant with virtues) (Thangamani *et al*., 2011). Banana (*Musa paradisiaca* L.), is the second largest fruit crop in India, belongs to family Musaceae, India ranks first in banana production, contributing about 23 per cent in world pool of banana production (Biswas and Kumar, 2010). Banana is a good source of calories, many other nutrients and enzymes. Fruit pulp contains vitamins B1, B2, B3, vitamin C, amino acids, iron, calcium phosphorus and proteins in substantial amount which are the daily need diet for human beings (Das, 2010).Bananas in storage and transit are subjected to various fungal rots (Sawant and Gawai,2011).

Worldwide postharvest losses in fruits range from 25 to 40 per cent in developed countries and upto 50 per cent in developing tropical countries (Hailu *et al*., 2013 and Bhale, 2011). Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients elements and their low pH values make them particularly desirable to fungal decayed (Bhale, 2011).

Even a small loss can be very expensive because of the accumulated cost of growing, harvesting and storing these high value commodities (Tripathi and Shukla, 2007). In India; Tamil Nadu, Maharashtra, Gujarat, A.P., Karnataka, Bihar, W.B., M.P., Assam and Kerala are the major banana growing states (Biswas and Kumar, 2010).

Realizing the need for analysis of annual losses of banana fruit in the region of eastern Uttar Pradesh

investigation was conducted in year 2015 in Gorakhpur. Gorakhpur is a terai region situated in eastern part of Uttar Pradesh (India) between latitude of 27°05' to 27°25' north and longitude of 83°20 to 84°10' east at elevations ranging from 107 m above sea level. High humidity and warm storage temperature favour the proliferation of several fungal species on Perishables which affect the retailer's earning. The investigations on post-harvest diseases of banana have remained confined to mere reporting of the diseases and its pathogens. Current study included an elaborate market study of Gorakhpur city (U.P.) regarding annual disease incidence in terms of per cent fruit spoiled by rotting pathogens and isolation, identification of post-harvest fungal pathogens of banana.

RESEARCH METHODOLOGY

Survey :

The survey was conducted during November 2014 to October 2015 in local fruit markets of Gorakhpur. The eight fruit markets *viz.*, A F market, Asuran, Bargadawa, Dharamshala, Kachari, Medical, Nausad and Raptinagar of Gorakhpur city were chosen and visited fortnightly for survey. Randomly selected retailer were visited for observation of disease incidence and sample collection of diseased fruits. From each market four to five banana retailers were considered for sampling. All infected fruits were stored in clean polyethylene bags with labels. The healthy samples were also stored separately for comparative and lab study.

Isolation of fungi :

Agar plate methods (*Muskett, 1948*) :

The infected tissue of fruits which showed typical symptoms were cut into small bits measuring about 6mm; surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution and washed repeatedly thrice with sterile distilled water to remove the traces of mercuric chloride. Then surface sterilized tissues were transferred to sterile Petri plates containing PDA (Potato dextrose Agar) (Himedia) medium under aseptic conditions. Petri plates were incubated in incubator at 28±2°C for seven days and fungal colony was observed by binocular microscope.

Standard blotter technique (*Tempe, 1953*) :

Three blotting papers were cut to size of Petri dish (140 mm); sterilized by70 per cent ethanol. These were allowed to dry and placed on to a pre sterilized Petri plate. The filter paper was moisten by 10-15 ml sterilized water. Surface sterilized fruits were dried between filter paper and plated on moisten blotter paper .The plates were incubated in incubator at (28±2º C) for seven days. After incubation the plates were observed under binocular microscope for fungal growth.

Identification of fungi:

Appeared fungal colonies were isolated, purified and maintained on potato dextrose agar medium. After purification of each fungal species, these were identified based on gross colony morphology and microscopic characters. Colony identification was based on colony characteristics such as colour, texture of mycelia and type of pigmentation. Detailed morphological characteristics of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single; the shape of spore etc. were identified with the help of fungal keys (Raper and Thom, 1949; Raper and Fennell, 1965; Booth, 1971 and Ellis, 1971). Pure cultures of the pathogens were maintained in the laboratory on PDA slants for further study. Pure fungal spore culture was maintained on PDA slants for 6-8 months at 4ºC±2ºC.

Pathogenicity test:

Pathogenicity test were conducted to confirm the pathogenic nature of isolated dominant mycoflora on banana fruit by method of Granger and Home (1924). Fruits of same size, maturity and preferably unblemished (no wound mark on surface) were taken for study. Three replicates were prepared from each fungus. The fruits were surface-sterilized by dipping them in 0.1 per cent mercuric chloride ($HgCl_2$) solution for two minutes. Thereafter, they were given five washing with sterilized distilled water so as to remove traces of $HgCl_2$. Three areas per fruit were marked on surface of fruit. A cork borer of 6mm diameter was flame sterilized and inserted into the banana fruit tissue (within the marked area). It was then taken out with a bit of tissue and the inoculum was placed in a pit. The piece of tissue taken out was inserted back to its position. All the inoculated fruits were incubated at room temperature (28±2ºC) and observed daily for symptom development.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized

under following heads :

Disease incidence of banana fruits during survey :

Disease incidence was evaluated by comparing the diseased fruit samples over total observed fruits in fruits markets of Gorakhpur. Data on incidence of different postharvest diseases were recorded when disease symptoms developed on the surface of ripened fruits. Disease incidence was calculated by the following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of infected fruits}}{\text{Total nmuber of fruits assessed}} \times 100$$

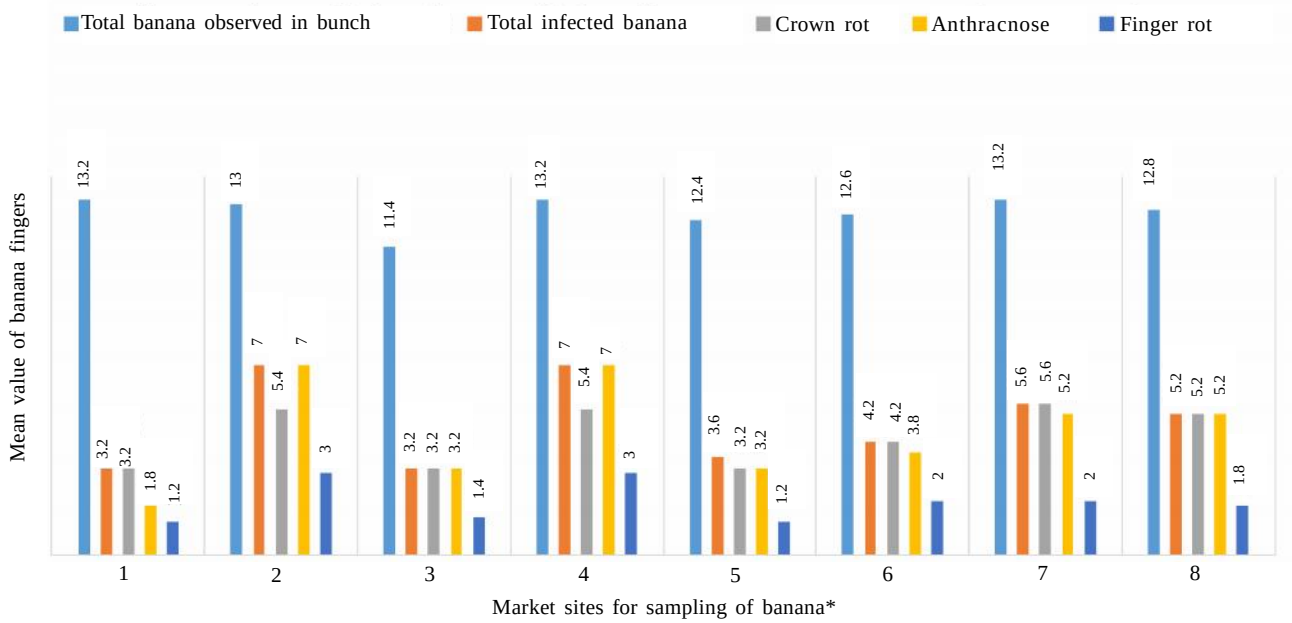
Table 1 showed overall evaluation of diseased samples present in every market site. It was found that per cent of disease incidence was 18 to 38.7 in local fruits market sites of Gorakhpur. Maximum per cent disease incidence was found in between July to October months in Bargadwa fruit market (38.7 %). The period of July to October lies in monsoon season having high humidity and high temperature. While minimum per cent disease incidence was found in

Table 1: Disease incidence during survey

Parameter / Place	I (Nov. 2014- Feb. 2015)				II (March 2015– June 2015)				III (July 2015 – Oct. 2015)			
	Total retailer visited	Total sample of banana observed	No. of banana infected	% of disease incidence	Total retailer visited	Total sample of banana observed	No. of banana infected	% of disease incidence	Total retailer visited	Total sample of banana observed	No. of banana infected	% of disease incidence
A.F Market	4	44	10	22.7	4	50	16	32.0	5	42	15	35.7
Asuran	4	52	11	21.1	5	62	20	32.2	5	46	17	36.9
Bargadwa	5	56	12	21.4	5	62	21	33.8	5	62	24	38.7
Dharmshala	5	60	14	23.3	5	58	20	34.4	4	62	23	37.0
Kachari	4	44	10	22.7	5	56	19	33.9	5	60	22	36.6
Medical	6	70	16	22.8	4	44	14	31.8	4	50	18	36.0
Nausad	4	52	12	23.0	5	54	17	31.4	4	42	16	38.0
Rapti Nagar	4	44	8	18.0	5	60	19	31.6	5	52	19	36.5

Table 2: Description of post-harvest diseases of banana

Crown rot		The cut end gives rise to profuse oozing which attracts pathogens from air; The infection of the cushion is initiated in the form of limited spotting, blemishes or extensive rotting extending to the finger-stalk and finger.
Anthracnose		Symptom occur on the body of the ripening fruit as almost circular dark brown areas with diffused edges which turn dark brownish to black color and become sunken. A mass of spores pink to rusty red color become pronounced in humid weather.
Finger rot		The pathogen is initially present at the tip immediately below the perianth or style, spread uniformly along the fruit causing a progressive brownish black discoloration and softening of the pulp.



***Market sites:** (1) A.F Market, (2) Asuran, (3) Bargadwa, (4) Dharmshala, (5) Kachari, (6) Medical, (7) Nausad, (8) Rapti Nagar

Fig. 1 : Prevalence of post-harvest diseases of banana in Gorakhpur city

Raptinagar between months of November to February (18.0 %). That period lies in winter season.

Description of post-harvest diseases of banana fruit:

Table 2 shows the frequently encountered market diseases during survey of Gorakhpur markets sites with their symptom on surface of fruiting body,description of diseases (crown rot, anthracnose and finger rot) are given in the table and occurrence of post-harvest diseases in every market sites shows in Fig. 1.

Crown rot starts at cut ends of crown region, that attracted by the pathogen. Later on whole banana crown portion get infected and get detached from fruiting body. Anthracnose appeared as dark brown circular lesion on peel. Further these lesion increases in size and finger rot as dark brown and black patches of discoloration present

Table 3: Isolated fungi from infected banana fruit samples

Pathogen	Disease	Symptoms
Aspergillus flavus Link	Crown rot	Dark brown to black, dry spot on fruit surface
A. niger Tiegh.	Crown rot	Dark brown to black, dry spot on surface.
Alternaria spp. (Fr.) Keissl	Crown rot	Brown circular spots increases and the lesions extend to the pulp in ripe fruits
Botryodiplodia theobromae Pat.	Crown rot, finger rot and anthracnose	Disease starts as brown water soaked lesion; at later stage rot advance as dark brown lesion with irregular margin
Colletotricum musae (Berk. and M.A. Curtis) Arx	Anthracnose and crown rot	Circular light brown spots enlarge and coalesce to form large dark brown spots.
Fusarium equiseti (Corda) Sacc	Crown rot	Circular or elongated dark-brown spots increase in size.
F. moniliforme J. Sheld	Crown rot	Discolored spot appears on fruit, later turned brown as lesion increases.
F. oxysporum Schltdl.	Crown rot	Circular or elongated dark brown spots increase in size.
Fusarium solani (Mart.) Sacc	Finger rot	A small raised yellow blemish increases and developed longitudinal crack, turning black.
Mucor circinelloides Tiegh.	Crown rot	Dark brown lesion appears on the surface of fruit
Penicillum spp.	Crown rot	Light brown water soaked spots later tissue releases watery exudates.
Rhizopus stolonifer (Ehrenb:Fr) Vuill.	Finger rot	Brown lesion with irregular margin increases and invades the fruit.

Plates of isolated fungi

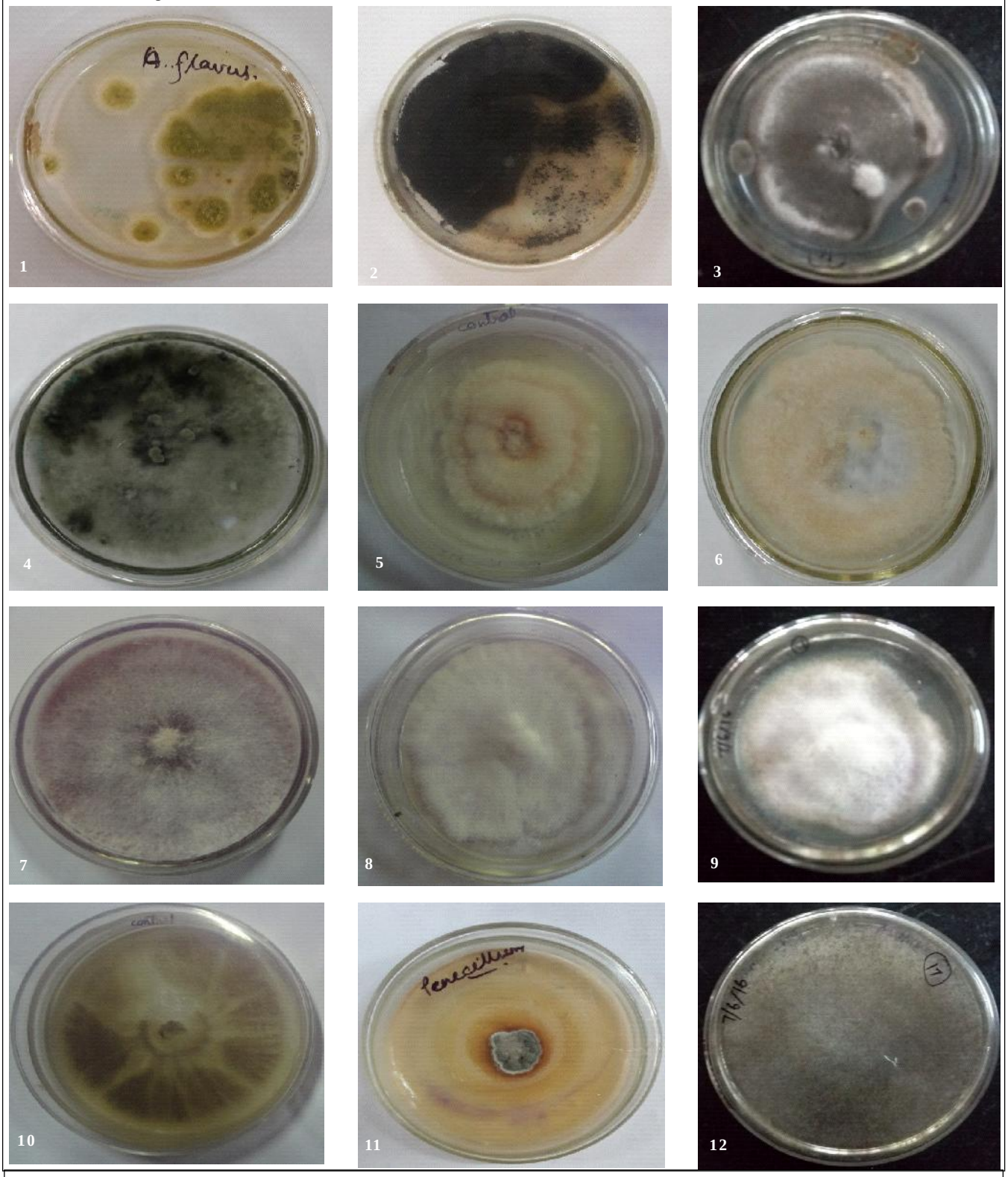


Fig. 2: **(1)** ***Aspergillus flavus,*** **(2)** ***A. niger,*** **(3)** ***Alternaria*** **spp., (4)** ***Botryodiplodia theobromae,*** **(5)** ***Colletotricum musae,*** **(6)** ***Fusarium equiseti,*** **(7)** ***Fusarium monoliformae,*** **(8)** ***Fusarium oxysporum,*** **(9)** ***Fusrium solani,*** **(10)** ***Mucor circinelloids,*** **(11)** ***Penicillum*** **spp., (12)** ***Rhizopus stolonifer***

in fruiting body. Later on softening and oozing of liquid from pulp was observed. The diseased samples of banana showed symptom of crown rot maximum followed by anthracnose and finger rot. Crown of fruit subjected to primary infection, as pathogens persist in crown portion of fruit, infection move on to fruit body and further caused anthracnose and finger rot.

Isolated fungi with their symptoms on infected banana fruit :

The fungi isolated from infected banana samples and their symptoms are shows in Table 3. Total 12 fungisolated from post-harvest banana diseases by agar plate method and standard blotter method shows in Fig. 2.

Crown rot disease is associated with more than one fungi *viz., Aspergillus flavus, A. niger, Alternaria*spp., *Botryodiplodia theobromae, Colletotricum musae, Fusarium equiseti, F. moniliforme, F. oxysporum, F. solani, Mucor circinelloides* and *Penicillum* spp. were isolated from crown portion of infected banana samples. *B. theobromae* and *C. musae* isolated from anthracnose disease (circular dark brown areas). *B. theobromae* and *Rhizopus stolonifera* were isolated from finger rot (dark brown lesion with irregular margin). Out of these fungi *B. theobromae* and *C.musae* were dominant, both fungi associated with crown rot, anthracnose and finger rot.

Pathogenicity :

Pathogenicity test for fulfilling the Koch's postulate was done on *B. theobromae* and *C. musae* shows in Fig. 3. These two fungi were found dominant among all fungi. Both fungi showed similar symptoms on artificial inoculums as observed under in natural infection. *B. theobromae* showed symptom as puffygrayish fungal colony grew; the colony growth rate was so high that whole banana fruit was covered with fungus. Later on colony was suppressed down and caused watery liquid exudation from fruit that caused softening of pulp and it became impropriate for consumption, while *C. musae* appeared as dark brown sunken spot, that later became large in size and spread all over the fruit.

This investigation embraces an extensive survey of local fruit markets of Gorakhpur for evaluation of disease incidence and the mycobiota associated with post-harvest rot of banana fruits. In this respect Abdullah *et al.* (2016) mentioned that fruits can affected by a wide range of micro-organisms such as fungi which have a serious threat to production of fruits. Spoilage attributes to any change in the condition of food making it less palatable or even toxic; these alterations may be accompanied by changes in taste, smell and appearance.

During the first part of this investigation, survey was focused on month wise disease incidence on local fruit markets. Banana is easily available throughout year. So its year round availability survey data assessed quarterly. Survey data assembled and evaluated in three parts November to February, March to June and July to October month. Disease incidence evaluated was

Control | *Colletotricum musae* | *Botryodiplodia theobromae*

Fig. 3 : Pathogenicity test

maximum between July to October month which indicate that banana fruit is more suseptible to pathogen infection in monsoon season having high humidity and temperature. While minimum disease incidence recorded between November to December month that indicate that in low temperature and less humidity pathogen severity become less. Singh *et al.* (2012) reported optimum temperature for rot ranged 17-30°C along with RH of 80 per cent. This explains the increased rotting during rainy season with optimum environment. Haque *et al*. (2003) also stated that anthracnose and finger rot highly observed between April to September in Bangladesh. They directly correlate the temperature with disease.

Observing disease symptom, crown rot with Anthracnose and finger rot were recorded. Crown rot infection start in crown portion that primarily initiation of post-harvest diseases later on Anthracnose disease appeared as brown circular spot, finger rot was also observed during survey.

Crown rot was complex disease, it was associated with more than one pathogen. About 12 pathogens were isolated from infected crown region. Anthracnose and finger rot caused by *C.musae* and *B.theobromae*, respectively. This isolation showed resemblance with many research outcomes (Lassois *et al.*, 2008; Gunasinghe *et al.*, 2004; Cruz *et al.*, 2013 and Diedhiou *et al.*, 2014).

Pathogenicity test also showed positive result for *C. musae* and *B. theobromae*. These both pathogen had similar symptom on artificial inoculum as present in natural condition of infection.

Conclusion:

The present study revealed the post-harvest deterioration of banana in Gorakhpur (UP) and reported the correlation of these diseases with fungus and climate. Mycoflora isolated from diseased banana released mycotoxin that created health hazard for human being and make banana unsuitable for consumption. So it is necessary to pay attention about enlisting pathogens, those causing post-harvest diseases and further control them by natural pesticides.

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LITERATURE CITED

Abdullah, Q., Mahmoud, A. and Al-harethi, A. (2016). Isolation and identification of fungal post-harvest rot of some fruits in yemen. *PSM Microbiol.*, **1**(1) : 36-44.

Bhale (2011). Survey of market storage diseases of some important fruits of Osmannabad district (M. S.) India. *Sci. Res. Report.*, **1**(2): 88-91.

Bilgrami, K.S., Jamaluddin, R. and Rizwi, M.A. (1979). *Fungi of India.* Today and Tomorrow Printers and Publishers, NEW DELHI, INDIA.

Biswas, B.C. and Kumar, L. (2010). High density planting: Success stories of banana farmers. *Fertil. Mktg. News*, **41** (6): 3-10.

Booth,C. (1971). *The genus Fusarium*. Common Wealth Mycological Institute Kew, Surrey, England.

Cruz, M.E.S., Schwan-Estrada, K.R.F., Clemente, E., ITako, A.T., Stangarlin, J.R. and Cruz, M.J.S. (2013). Plant extracts for controlling the post-harvest anthracnose of banana fruit. *Rev. Bras. Pl. Med., Campinas,* **15** (4) : 727-733.

Das, J.L. (2010). Medicinal and nutritional values of banana cv. NENDRAN. *Asian J. Hort.*, **5**(1): 11- 14.

Diedhiou, M.P., Zakari, H.A., Mbaye, N., Rokhaya, F. and Samb, L.P. (2014). Control methods for postharvest diseases of banana (*Musa sinensis*) produced in Senegal. *Int. J. Sci. Environ. Technol.*, **3**:1648-1656.

Ellis, M.B.(1971). *Dematiaceous Hyphomycetes.* Common wealth Mycological Institute, Kew, Surrey, England.

Ellis, M.B.(1976). *More dematiaceous hyphomycetes.* Common wealth Mycological Institute, Kew, Surrey, England.

Gilman, J.C.(1998). *A manual of soil fungi*. Biotech Books, NEW DELHI, INDIA.

Granger, K. and Home, A.S. (1924). A method of inoculating the apple. *Ann. Bot.*, **38**: 212-215.

Gunasinghe, R.N., Ikiriwatte, C.J. and Karunaratne, A.M. (2004). The use of Pantoea agglomerans and *Flavobacterium* sp. to control banana pathogens. *J. Hort. Sci. Biotechnol.*, **79** :1002–1006.

Hailu, M., Workneh, T.S. and Belew, D.(2013). Review on postharvest technology of banana fruit. *African J. Biotechnol.*, **12** (7) : 635-647.

Haque, M.A., Khalequzzaman, K.M., Islam, M.S. and Hossain, M.M. (2003). Survey the prevalence of market diseases of banana. *Pakistan J. Plant Pathol.*, **2** (3) : 169-173.

Kader, A.A.(2013). Postharvest technology of horticultural crops - an overview from farm to fork. ethiop. *J. Appl. Sci. Technol.*, **(1)**: 1- 8.

Lassois, L., de Lapeyre, de Bellaire, L. and Jijakli, M.H. (2008). Biological control of crown rot of bananas with *Pichiaanom alastrain* K and *Candida oleophila* strain. *Biological Control,* **45**(3): 410- 418.

Muskett, A.E. (1948). Technique for the examination of seeds for the presence of seed borne fungi. *Trans. Brit. Mycol. Soc*., **30**:74-83.

Nath, K., Solanky, K.U. and Bala, M.(2015). Management of banana (*Musa Paradisiaca* 1 L.) fruit rot diseases using fungicides. *J. Plant Pathol. Microb.*, **6**: 6-8.

Pushpangadan, P., Kaur, J. and Sharma, J.(1989). Plantain or edible banana (*Musa x paradisica var – sapiemtum*) some lesser known folk uses in India. *Ancient Sci. Life,* **9** (1) : 20-24.

Qais, A., Ahmed, M. and Amira, A.(2016). Isolation and identification of fungal post-harvest rot of some fruits in yemen. *PSM Microbiol.,***1** (1) : 36-44.

Raper, K.B. and Thom, C.(1949). *A manual of Penicillia*. Willium and Wilkins, Baltimore.

Raper, K.B. and Fennell, D.I. (1965). *The genus Aspergillus.* Willium and Wilkins, Baltimore.

Sawant, S.G. and Gawai, D.U. (2011). Biochemical changes in banana fruits due to postharvest fungal pathogens. *Curr. Bot.*, **2** (1) : 41-42.

Singh K., Nizam S., Sinha M., Verma P. K. (2012). Comparative transcriptome analysis of the necrotrophic fungus Ascochyta rabiei during oxidative stress: insight for fungal survival in the host plant. *PLoS ONE*, **7**:e33128.

Sutton, B.C.(1980). *The coelomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England.

Tempe, De (1953). The blotter method of seed health testing. *Proc. Int. Seed Test Ass.*, **28**:133-151.

Thangamani, P.R., Kuppusamy, P., Faisal, M., Gandhi, P.K. and Raguchander, T. (2011). Morphological and physiological characterization of *Colletotrichum musae* the causal organism of banana anthracnose. *World J. Agric. Sci.,***7** (6) : 743-754.

Tripathi, P. and Shukla, A.K. (2007). Emerging non-conventional technologies for control of post-harvest diseases of perishables. *Fresh Prod.,***1** (2) : 111-120.