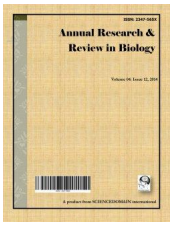


Annual Research & Review in Biology

26(6): 1-7, 2018; Article no.ARRB.41804
ISSN: 2347-565X, NLM ID: 101632869

Isolation, Identification and Pathogenicity of Fungal Organisms Causing Postharvest Spoilage of Tomato Fruits during Storage

Liamngee Kator[1*], A. C. Iheanacho[2] and Kortse P. Aloho[3]

[1]Department of Biological Sciences, Benue State University Makurdi, Benue State, Nigeria.
[2]Department of Agribusiness, University of Agriculture Makurdi, Benue State, Nigeria.
[3]Department of Plant Breeding and Seed Science, University of Agriculture Makurdi, Benue State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author LK designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors ACI and KPA managed the analyses of the study. Author KPA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/41804
Editor(s):
(1) Jin-Zhi Zhang, Key Laboratory of Horticultural Plant Biology (Ministry of Education), College of Horticulture and Forestry Science, Huazhong Agricultural University, China.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Nneoma Confidence .J. Anyanwu, COMSATS University, Pakistan.
(2) Venkata Satish Kuchi, Dr. YSR Horticultural University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/24853

Original Research Article

Received 15th March 2018
Accepted 23rd May 2018
Published 28th May 2018

ABSTRACT

Isolation, identification and pathogenicity of fungal organisms causing postharvest spoilage of tomato fruits during storage was carried out. Tomato fruits showing symptoms of rot were collected from the store house. Small sizes were cut and surface sterilized in 1% of Sodium hydrochloride and rinsed in several changes of sterile distilled water. They were plated on Potato Dextrose Agar (PDA) and observed for fungal growth. Identification was done macroscopically and microscopically. For pathogenicity, healthy tomato fruits were plugged with pure cultures of the fungal isolates and disease incidence and severity were evaluated. Five fungi namely *Aspergillus flavus, Penicillium waksmanii, Botryodiplodia theobromae*, *Fusarium oxysporum* and *Colletotrichum asianum* were isolated. Incidence of decay on healthy tomato fruits

**Corresponding author: E-mail: katorliamgee@gmail.com;*

was 100% for all fungal isolates while the control was 0%. T-test revealed significant differences between the inoculated and the controls at 1% and 5% levels of probability. Severity of decay ranged from 51–53% for all fungal isolates, while the controls showed 0%. T-test revealed significant differences between the inoculated and the control at 1% and 5% levels of probability. Pathogenic microorganisms on tomato are a potential health hazard to man and animals following ingestion.

Keywords: Postharvest; storage; tomato; isolation; spoilage; fungi.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum L.*) which belongs to the family of the *Solanaceae* is one of the most widely grown and extensively consumed vegetable in the world [1]. It is considered an important cash and industrial crop in many parts of the World [2]. Tomato can be consumed in a number of ways and in countless number of dishes. The fresh tomato fruits are eaten often raw or as an ingredient in salad and sandwiches. The processed ones are consumed dried or as paste, soup, stew and are used for producing drinks [3]. It is rich in vitamins, minerals, lycopene, sodium, iron, phosphorus, β-carotene, potassium and magnesium [4]. In the Nigerian Savanna, fresh tomato is the most valuable vegetable crop. It accounts for about 18% of the average daily consumption of vegetables in Nigeria. Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic. These changes may be accompanied by alteration in taste, smell, appearance or texture [5]. Fungi are the most important and prevalent pathogens, infecting a wide range of fruits and causing destructive and economically important losses of the fruits during storage, transportation and marketing [6]. Therefore, the objective of this study was to isolate and identify fungal pathogens causing postharvest spoilage of tomato fruits during storage.

2. MATERIALS AND METHODS

2.1 Preparation of Potato Dextrose Agar (PDA)

The medium used for isolation of fungal microorganisms was Potato Dextrose Agar (PDA). PDA was prepared by dissolving 40 grams of media in 1000 ml of sterile distilled water. The solution was heated on a heating mantle to dissolve the medium completely. The medium was then sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes, after which it was removed and allowed to cool before pouring into sterile Petri dishes.

2.2 Isolation of Fungal Organisms from Decaying Tomato Fruits during Storage

Tomato fruits showing symptoms of rot were collected during the storage period for isolation of fungal microorganisms. Small sizes were cut from the tomato fruits infected with rot and surfaced sterilized by dipping in 1% sodium hypochloride (NaOCl) solution for thirty seconds. They were removed and rinsed in several changes of sterile distilled water and placed on solidified Potato Dextrose Agar (PDA) medium. Three replicates were made for each sample. The inoculated plates were incubated at room temperature and observations were made for microbial growth. After 5–7 days of growth, subculturing was done to obtain pure cultures of the isolates as reported by Liamngee et al. [7]. To subculture the fungal isolates, a wire loop was flame sterilized and used to scoop out a little quantity of each pathogen and inoculated in another Petri dish containing solidified PDA. The loop was used to streak the inoculums on the PDA and left to grow. The plates were sealed with PVC tapes to avoid contamination. Plates were incubated for 5–7 days at room temperature.

2.3 Identification of Fungi

Identification was done macroscopically and microscopically. For macroscopic identification, colony characteristics such as appearance, change in medium colour and growth rate were observed on the Petri plates. For microscopic identification, a thin smear of fungi isolates from 5–7 day old cultures were inoculated aseptically on a clean glass slide using a sterile inoculating loop. A drop of lactophenol cotton blue was added and the mixture was covered with a cover slip and viewed under 40x objective of the light microscope. Shapes of the conidia and conidiophores were taken note of. These

features were matched with standards described by Barnett and Hunter [8] and Booth [9] as reported by Liamngee et al. [7].

2.4 Pathogenicity Studies

The method reported by Liamngee et al. [7] was used. Healthy tomato fruits were washed with distilled water and thereafter sterilized in 1% Sodium hypochloride solution for thirty seconds. Mycelia discs of fungal isolates from five day old cultures were used to inoculate the tomato fruits. The cylindrical plugs (5 mm) were used to plug holes created in the tomato fruits by a cork borer. The discs of the tomato fruits in the cork borer were replaced and sealed with sterile PDA. On appearance of symptoms, the tissues at the margin of the healthy and diseased parts were excised, sterilized and placed on PDA and incubated at room temperature for 5–7 days. At the end of this period, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates. Three tomato fruits were used for each fungal isolate, replicated three times and arranged in complete randomized design. Controls were tomato fruits inoculated with sterile PDA only. After 5–6 days of post inoculation, hand feel and visual examination of the exterior and interior of the fruits were used to ascertain the symptoms of fruit rots. Disease incidence and severity on the tomato fruits were determined. Disease severity was determined by applying the rating scale of [10] with slight modification in which 0 = no disease symptom, 1 = 1 – 20% severity level on infected fruits, 2 = 21 – 40%, 3 = 41 - 60%, 4 = 61 – 80% and 5 = 81 – 100%. These were applied in the formula proposed by Akhtar and Alam [11].

Disease incidence % (DI) = $X/N \times 100$

Where X= number of infected fruits and N = total number of fruits sampled.

Disease severity % (DS) = $\Sigma (a + b) / N.Z \times 100$

Where, $\Sigma (a + b)$ = Sum of symptomatic fruits and their corresponding score scale, N = Total number of fruits sampled and Z = highest score scale.

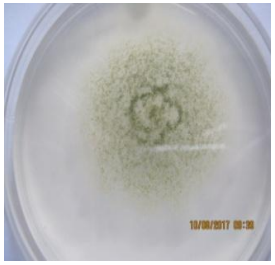
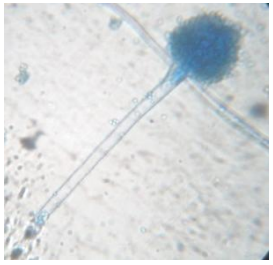
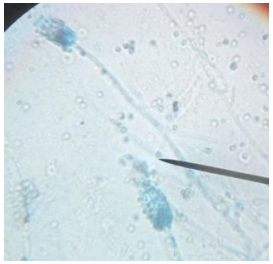
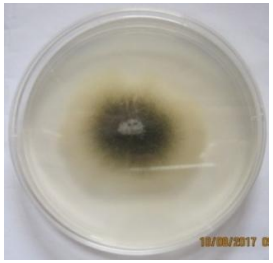
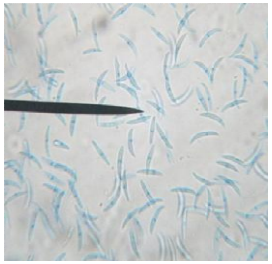
3. RESULTS AND DISCUSSION

The fungal organisms isolated and identified from decaying tomato fruits during the storage period were *Aspergillus flavus, Penicillium waksmanii, Botryodiplodia theobromae*, *Fusarium oxysporum* and *Colletotrichum asianum*. Some of these organisms have been previously reported as pathogens of tomato fruits by Ogo-oluwa and Liamngee [12]. They isolated and identified *Aspergillus niger* and *Fusarium spp* from decaying tomato fruits. Also, Ijato *et al.* [13] isolated *Aspergillus niger* and *Fusarium spp* from rotting tomato fruits.

Olaniran et al. [14] also isolated *Aspergillus niger, Fusarium species* and *Botryodiplodia theobromae* from orange fruits. Also, Liamngee et al. [7] isolated *Aspergillus flavus*, *Botryodiplodia theobromae*, *Aspergillus niger* and *Aspergillus fumigatus* from decaying yam tubers in storage. Different fungal species such as *Alternaria*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus*, *Aspergillus and Colletotrichum* have been implicated as Spoilage organisms [6]. Other fungi reported to be associated with postharvest rot of tomato include *Aspergillus flavus, Fusarium solani, Monilocaetes infuscaus, Penicillum spp, Certolystis finbriatia, Diapoc batatalis* [15]. Fungi are the most important and prevalent pathogens, infecting a wide range of fruits and causing destructive and economically important losses of fruits during storage, transportation and marketing [16].

The pathogenicity test result revealed that all the fungal isolates identified had the ability to cause infection in healthy tomato fruits, though at various percentages of severity. The healthy tomato fruits showed symptoms of rot after Five (5) days of inoculation with fungi mycelia. Morphological and microscopic characteristics were compared with initial cultures after re-isolation and were found to be the same. The controls (healthy tomato fruits inoculated with sterile PDA only) showed no symptom of rot. T-test revealed significant difference in the pathogenicity of the healthy tomato fruits between the inoculated and Control at 1% and 5% levels of significance. This agrees with the work of Chuku et al. [17] on the pathogenicity of fungal isolates on tomato fruits. The ability of the fungal isolates to cause infection in healthy tomato fruits was due to the fact that the pathogens are able to utilize the nutrients of the fruits as a substrate for growth and development [7].

Table 1. Characterization of fungal isolates from decaying tomato fruits during storage

Macroscopic characteristics	Microscopic characteristics	Appearance on PDA	Photomicrograph	Probable organism
The surface colour of the colony is greyish green and the margins are entire. The reverse is hyaline. The colony growth is moderate to rapid	The vesicle is hemispherical, Conidia is globose (biseriate) covering the entire vesicle. Conidiophore is smooth, long and hyaline.			***Aspergillus flavus***
The colour of the colony is dark-green to greyish green, powdery and compact with a white border. The reverse is light orange and the growth rate is moderate.	Conidia are unicellular, round to ovoid, smooth and in chains. Phialides are grouped in brush like clusters (Penicilli) at the ends of conidiophores which are smooth.			***Penicillium waksmanii***
Initially, the colony colour was white which changed to greyish black in colour with a fluffy aerial growth. The reverse side of the colony is black in colour. Growth rate is moderate.	Conidia are thick-walled and oval in shape. Each conidium have a septum			***Botryodiplodia theobromae***

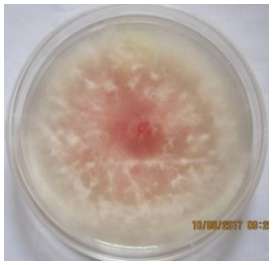
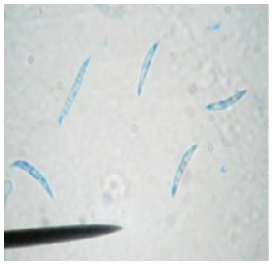
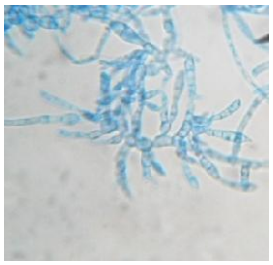
Macroscopic characteristics	Microscopic characteristics	Appearance on PDA	Photomicrograph	Probable organism
The colour of the colony is purple with a cottony mycelium and a dark purple under surface The reverse colour is red to purple. Growth is 5 – 6 days.	Macroconidia have 2-3 septa and are oval and tapering. Chlamydospores are produced on the hyphae. Microconidia are present.			***Fusarium oxysporum***
The colony colour is white with a cottony mycelium which becomes orange with age. On the reverse, the colour was orange and becomes brown with age.	Presence of hyaline conidiophores. Conidia is hyaline with fusiform to slightly rounded ends.			***Colletotrichum asianum***

Table 2. Incidence of decay on healthy tomato fruits inoculated with the test fungi.

Treatment	*Aspergillus flavus*	*Penicillium waksmanii*	*Botryodiplodia theobromae*	*Fusarium oxysporum*	*Colletotrichum asianum*
Inoculated	100.00	100.00	100.00	100.00	100.00
Control	0.00	0.00	0.00	0.00	0.00
T-test (0.05)	**3.46****	**3.46****	**3.46****	**3.46****	**3.46****

** *significant at 1% and 5% level of probability*

Table 3. Severity of decay on healthy tomato fruits inoculated with test fungi

Treatment	*Aspergillus flavus*	*Penicillium waksmanii*	*Botryodiplodia theobromae*	*Fusarium oxysporum*	*Colletotrichum asianum*
Inoculated	51.00	53.00	53.00	53.00	53.00
Control	0.00	0.00	0.00	0.00	0.00
T-test (0.05)	**25.50****	**30.00****	**30.00****	**30.00****	**30.00****

** *significant at 1% and 5% level of probability*

Fruits due to their low pH, high moisture content and nutrients composition are very susceptible to attack by pathogenic fungi which in addition to causing rots, also make them unfit for consumption by producing mycotoxins. The contamination of the tomato fruits by these fungal pathogens could be as a result of poor handling practices in the tomato production chain, storage conditions, distribution and changing physiological state of the fruits [16].

4. CONCLUSION

This study revealed that several fungi are responsible for postharvest decay of tomato fruits during storage. Pathogenic microorganisms on tomato are a source of potential health hazard to man and animals following ingestion. This is due to their production of toxins which are capable of causing disease. Therefore, the Hazard Analysis Critical Control Points (HACCP) should be employed at every point in the postharvest chain to minimize contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Grandillo S, Ku HM, Tanksley SD. Identifying loci responsible for natural variation in fruit size and shape of tomato. Theoretical application of genetics. 1999;99:978-979.
2. Babalola DA, Makinde YO, Omonona BT, Oyekanmi MO. Determinant of postharvest lossess in tomato production; A case study of Imeke Afon Local Government Area of Ogun State. Journal of Life and Physical Sciences, Acta Satech. 2010;3(2):14-15
3. Alam T, Goyal GK. Packing and storage of tomato puree and paste. Interbnational Journal for reviews in postharvest Biology and Technology. 2007;5:1-2.
4. Passam HC, Karapanes IC, Bebeli PJ, Savvas D. A review of recent research on tomato nutrition, breeding and postharvest technology with reference to fruit quality. European Journal of Plant Science and Biotechnology. 2007;1(1):1-3.
5. Berdegue JA, Balsevich F, Flores L, Reardon T. Central American supermarkets private standards of quality and saftety in procurement of fresh fruits and vegetables. Food policy. 2005;30:254-269.
6. Sommer NF, Fortingae RJ, Edwards DC. Postharvest diseases of selected commodities (Kader A.A. Ed). University of California Division of natural resources Publication. 1992;31:117-160.
7. Liamngee K, Akomaye MU, Okoro JK. Efficacy of some Botanicals in the Control of Fungi causing Postharvest rot of yam in Katube Market, Obudu, Nigeria. IOSR Journal of Pharmacy and Biological sciences. 2015;10(6)Ver II:33-41.
8. Barnett HL, Hinter BB. Illustration genera of imperfect fungi, 3Rd edition, Burgess Publing Co. Minnesoth, USA. 1972;273.
9. Broth C. The genus Fusarium, Survey, UK: CMI, Kew. 1971;237-238.
10. Bowen KL. Plant disease Epidemology In: Trigiano, RN, Windhan, NT, Windham, AS (eds), Plant Pathology, Concepts and

Laboratory exercises, 2nd Edn. CRC press, NY. 2004;281-283.
11. Akhtar KP, Alam SS. Assessment keys for some important disease of mango. Pakistan Journal Biological Sciences. 2007;5:246-250.
12. Aondo TO, Liamngee K. Evaluation of bitter leaf (*Vernonia amygdalina*) extract in the inhibition of fungi causing postharvest rot of tomato fruits in Makurdi, Benue state Nigeria. Der Pharmacia Lettre. 2016;11:69-73.
13. Ijato J, Otoide J, Ijadunola J, Aladejimokun A. Efficacy of antimicrobial effect of *Vernonia amygdalina* and tridax procumbens in Invitro control of tomato (*Lycopersicon esculentum*) postharvest fruit rot. Report and Opinion. 2011; 3(1):120-123.
14. Olaniran O, Ojo O, Odelade K. Survey of the Postharvest diseases Aflotoxin contamination of marketed orange fruit (*Citrus spp.*) in Major cities in Oyo state, Nigeria. Journal of Agriculture and Veterinary Science. 2014;7(8):69-73.
15. Snowdow AL. A colour atlas of postharvest disease and disorders of fruits and Vegetables. Wolfe scientific Ltd. London. 1990;1:672.
16. Beuchat LR. Vectors and Conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric disease. Britain Food Journal. 2006;108:38-53.
17. Chuku EC, Osakwe JA, Daddy-West C. Fungal Spoilage of Tomato (*Lycopersicon esculentum* Mill.) using ginger and garlic Scientia Africana. 2010;9(2):41.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24853