Original Research Article

Assessment of post-harvest pathogens of Khasi mandarin (*Citrus reticulata*, Blanco) from Meghalaya and evaluation of most potent artificial inoculation method to initiate *Penicillium* rot

ABSTRACT

Khasi mandarin is a unique germplasm of North East India owing to its exceptional juice quality, taste and aroma. But the shelf life of the fruit is decreasing due to the post-harvest decay pathogens. The present investigation was carried out in 2023-2024 and 2024-2025 with an aim to investigate post-harvest storage pathogens associated with Khasi mandarin in Meghalaya and to evaluate the most effective method of inoculation to get the highest infection. *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp., *Geotrichum,* and Mucor species were isolated as post -harvest associated pathogens with a maximum frequency of 71.42% against *Penicillium* spp. followed by *Fusarium* spp. (57.15%). Isolated pathogens were characterized morphologically and culturally. *Penicillium italicum,* being the most dominant post-harvest pathogen of Khasi mandarin, as proved by the pathogenicity test, was artificially inoculated by four different methods in the fully ripened mandarins and the most efficient inoculation method for disease development was evaluated by calculating disease severity index (DSI) which was found to be highest (83.33%) in the disc inoculation method, followed by injection (58.33%) and immersion (41.66%). Percent seed infection was also calculated by blotter test and found to be highest in disc inoculation method (96%) and lowest in spraying (76%) on 10th day of inoculation. The present investigation evaluates the post-harvest associated mycoflora of Khasi mandarin in Meghalaya and the most potent artificial inoculation method responsible for *Penicillium* rot.

Keywords: Blotter test, disc inoculation, disease severity index, Penicillium italicum

1.INTRODUCTION

Khasi mandarin or *soh-niamtra* (*citrus reticulata*, blanco) is a dominant cultivated citrus species of north east india, along with other citrus species like *citrus jumbhiri, citrus indica, citrus latipase* etc. it is mainly cultivated in meghalaya, west bengal, arunachal pradesh, sikkim, nagaland, tripura, manipur and assam (kumar *et al*., 2024). high juice content of 43.33ml to 46.82ml (borah *et al*., 2023) with unique taste and aroma makes the fruit a potent exporter from north east india. it is well established that the *state of meghalaya is the origin or primary gene center of khasi mandarin* (*citrus reticulata* family: rutaceae), cultivating mainly in east and west khasi hills, ri-bhoi, garo hills and jaintia hills (https://nhb.gov.in/). the fruit, khasi mandarin was granted for geographical indication (gi) tag in 2014 under the geographical indication of good, registration and protection act, 1988 of the government of india (doki *et al*., 2019) as the climatic condition for meghalaya is found to be more suitable for production of khasi mandarin as compared to contributing states. but, it deteriorates rapidly after harvest due to microbial infection. enormous nutrients with higher water content (80-89%) makes the fruit more vulnerable to many post-harvest pathogens. the prevailing high humidity is also an added advantage for post-harvest pathogen attack. due to which off-season availability is very less. limited work has been carried out on evaluating post-harvest pathogens associated with khasi mandarin in north east india that

are responsible for short shelf life of the fruit. to diagnose the most potent post-harvest pathogens associated with khasi mandarin and to evaluate the most efficient artificial inoculation methods, that leads to *penicllium* rot, the present investigation was carried out in 2023-2024 and 2024-2025 at college of post graduate studies in agricultural sciences, Umiam, Meghalaya, India

2.METHODOLOGY

2.1 COLLECTION OF SAMPLES

Infected Khasi mandarin fruits were collected from the local market of Ri Bhoi district of Meghalaya which is one of the dominating areas for Khasi mandarin production in Meghalaya. All total of 50 samples were collected in clean polypropylene bags with the dimensions of 10''×8'' and were brought to the Plant Pathology Laboratory at CPGS-AS, Umiam, Meghalaya for further processing.

2.2 ISOLATION AND PURIFICATION OF PUTATIVE PATHOGENS

The collected samples were double-washed with clean running tap water. Tissue segments (3-5 mm) containing both infected and diseased tissues were cut with a sterile flamed blade, and cut portions were surface sterilized with 1% NaOCl followed by three successive changes of sterile distilled water, and air dried the sample. Dried segments were placed equidistantly in previously prepared sterilized PDA plates and incubated at 25±1 ºC for 5 days under 12 hour photoperiod. Developed fungal colonies were purified by hyphal tip culture. The isolated putative pathogens were identified up to the generic level based on morphological and cultural characterization. The purified culture was maintained in PDA slants for further studies.

Purified fungal colonies were first identified by observing colony morphology, pigmentation, shape and structure of mycelium, branching pattern, presence of conidiophores, sclerotia, shape of conidia and were culturally characterized with Royal Horticultural Society Colour chart. Identification up to the generic level was made by consulting relevant literature (Pitt and Hocking, 1997; Samson *et al.,* 2004).

2.3 PATHOGENICITY TEST

Koch's postulates were carried out to find the most dominant species amongst the isolated post-harvest pathogens. Khasi mandarin fruits free from infection with equal size, texture and colour were selected for *in vitro* pathogenicity test and were carried out according to the Koch's postulates. The most dominant post-harvest fungal pathogen proved by *in vitro* pathogenicity test was further molecularly characterized and identified.

2.4 INOCULATION OF DOMINATE PATHOGEN WITH FOUR DIFFERENT INOCULATION METHODS

The most dominant *Penicillium* species confirmed from the pathogenicity test was inoculated with four different inoculation methods viz., spraying, immersion injection and disc inoculation method. Spore concentration of $2.65X10^6$ CFU/mL was used for spraying, immersion as well as injection method. Freshly prepared spore suspension was sprayed on the fully ripened equal size fruits with the help of hand held atomizer with the pore size of 0.8 mm till the run off condition and the fruits were kept in pre sterilized tray. For immersion method of inoculation, fruits were dipped in 250 ml conidial suspension with the above mentioned spore concentration for a period of 1 minutes and air dried the inoculated sample

and kept in sterilized tray. For injection method of inoculation, spore suspension were directly inserted into the skin of the sterilized fruits in four equidistant position and injected till run off condition. Inoculated mandarins were kept in pre sterilized tray. For disc inoculation, 6 mm disc from freshly prepared culture plates were inoculated with the help of a sterile cork borer in four equidistant positions of Khasi mandarin fruits. The inoculated fruits were kept in a pre-sterilized plastic tray and all the trays were covered with a perforated transparent polythene sheet and incubated at room temperature (28±1ºC). The fruits were observed each day for the appearance of visible symptoms.

2.5 ESTIMATION OF DISEASE SEVERITY INDEX (DSI)

The per cent disease severity index was calculated with the following disease rating scale

Grade	Range of infection
0	No infection
1	1-25% of the surface area of the fruit showing disease symptom
2	26-50% of the surface area of the fruit showing disease symptom
3	51-75% of the surface area of the fruit showing disease symptoms.
4	>75% of the surface area of the fruit showing disease symptoms.

and the disease severity index (DSI) was calculated on 10^{h} day of inoculation in each of the inoculation methods with the following formula

$$DSI(\%) = \frac{\Sigma((No.\ of\ diseased\ fruit\ in\ each\ rating\ category\ \ X\ Severity\ rating)}{Total\ No.\ of\ fruit\ assessed\ X\ Highest\ Scale}\ X100$$

The inoculated mandarins were also observed for pulp and seed infection, if any to investigate whether the infection was moved to the pulp and seed. Each of the segments from the inoculated mandarins was observed for *Penicillium* infection by removing the outer peel and evaluating the condition of each segment of the fruit by observing its compactness, colour and texture.

2.6 ESTIMATION OF PERCENT SEED INFECTION (%)

To evaluate the seed infection, the standard blotter test method was followed as per the ISTA (International Seed Testing Association) guidelines. Twenty-five numbers of Khasi mandarin seeds extracted from the inoculated fruits were plated in sterilized 9 cm petri plates with three layers of moistened filter papers soaked in sterile water and incubated the plates for 7 days. Control plates were maintained by seeds collected from the uninoculated mandarins. The incubated seeds were observed with magnification for the association of pathogens, if any. Four replications for each of the treatments were maintained. The percent seed infection was calculated by the following formula.

$$Percent\ seed\ infection\ (\%) = \left(\frac{(Number\ of\ infected\ seed)}{Total\ number\ of\ seed\ per\ plate}\right) \times 100$$

The fruits were weighed before inoculation and after inoculation of the pathogen. The findings were estimated as the percentage of weight loss with the following formula [8a].

$$Weight\ loss(\%) = \left(\frac{Wi - Ws)}{Wi}\right) \times 100$$

Where,
Wi= Initial fruit weight
Ws= Fruit weight at sampling date

3. RESULTS AND DISCUSSION

Ten (10) different fungal species belonging to five genera were found to be associated with the post-harvest deterioration of the Khasi mandarin. The symptoms on each fruit were recorded (Plate 1: a-h) before further processing. The isolated pathogens were identified based on morphological and cultural characteristics. Table 1, Plate 3(a-l) represents the cultural, morphological as well as microscopical characteristics of the isolated pathogen from the Khasi mandarin and these characteristics were recorded as per the Royal Horticultural Society Colour Chart (Table 3) (Plate 2a-f). While evaluating the frequency of occurrence (Table 2) of post-harvest pathogen, *Penicillium* spp. being the most frequently occurring pathogen (71.42%) followed by *Fusarium* spp. (57.14), *Aspergillus* niger (42.85%) and Mucor spp (42.85%) (Fig.1). The most dominant post-harvest pathogen of Khasi mandarin was identified with molecular characterization and found 100% similarity with *Penicillium italicum* PV931913).

Table 1: Cultural, morphological, and microscopical characters of the isolated pathogens from Khasi mandarin

Pathogen isolated	Cultural characters	Microscopic characters
Aspergillus flavus	Initially whitish mycelia, later yellowish green, periphery irregular, fluffy, zonate in Potato Dextrose Agar (PDA) media.	Conidia are olive green in colour in (PDA)
Aspergillus niger	Initially, the colour of the mycelia is white, later turns black quickly in PDA	Aerial conidia are developed, margin irregular, conidia always in chains
*Muc*or spp.	Brownish grey colonies in PDA, floccose, growing repeatedly in PDA	Mycelium coenocytic, globular sporangia
Geotrichum spp.	Whitish mycelia, equally spreading, fast growing in PDA	Hyphae are fragmented into single cells, smooth arthroconidia
Fusarium spp. 1	Moderate growing in PDA, initially white, later on dark pinkish, reverse reddish in colour.	Aerial mycelium, producing macroconidia
Fusarium spp. 2	Moderate growing in PDA, initially white, later on light pinkish, reverse light pinkish in colour.	Aerial mycelium, microconidia are produced.
Penicillium spp. 1	Yellowish green flat colonies with irregular periphery, center green. Periphery white in Czapex Dox Agar (CDA) medium, reverse dark brownish green.	Conidia are oval, mycelium is septate
Penicillium spp. 2	Conies are scattered, with a whitish margin, dark green colour, and reverse whitish-yellow colour.	Conidia are oval, mycelium is septate
Penicillium spp. 3	Dark green colonies, reverse brown, slow growth	Conidia are spherical
Penicillium spp. 4	Dark small green scattered colonies with regular margins, reverse yellowish brown	Conidia are spherical, hyaline



1 (a) Brown dry rot near stem end, initially starting from the stalk end and later spreading on the skin. Shrinkage of the discoloured skin.

(b) Brown soft rot in oval-shaped manner.



©Multiple dot spot coalaces, spots are black in colour, sooty mold like growth observed.

(d) Blackish brown irregular spot deepseated, coalacing, average spot diameter is 0.26 cm.

(e) Brownish discolouration and white mycellial growth.

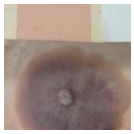


(f) Blueish green mycellial growth with blackish discolouration.

(g) White mycellial growth near stalk end, softing of tissue near stalk end rot.

(h) White irregular mycellial growth with brownish discolouration.

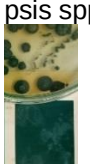
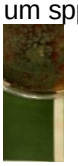
Plate 1(a-h): Symptoms observed from the sample collected for isolation of post-harvest associated pathogen.



2(a)Fusarium spp.

2(b)Pestalotiopsis spp.

2(c)Penicillium spp.

2(d)Penicillium spp.

2(e)Penicillium spp.

2(f)Penicillium spp.

Plate. 2 (a-f) Culturally characterized isolated spp. with Royal Horticultural Society Colour Chart

Fig 1. Frequency of occurrence of the Postharvest pathogen of Khasi mandarin

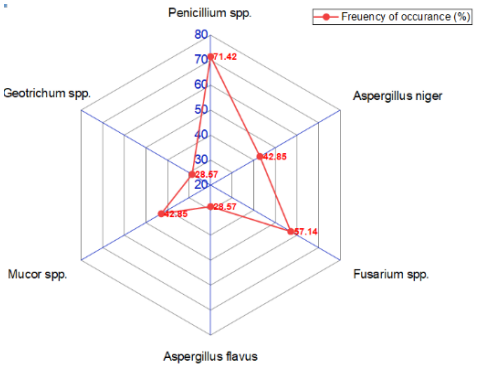
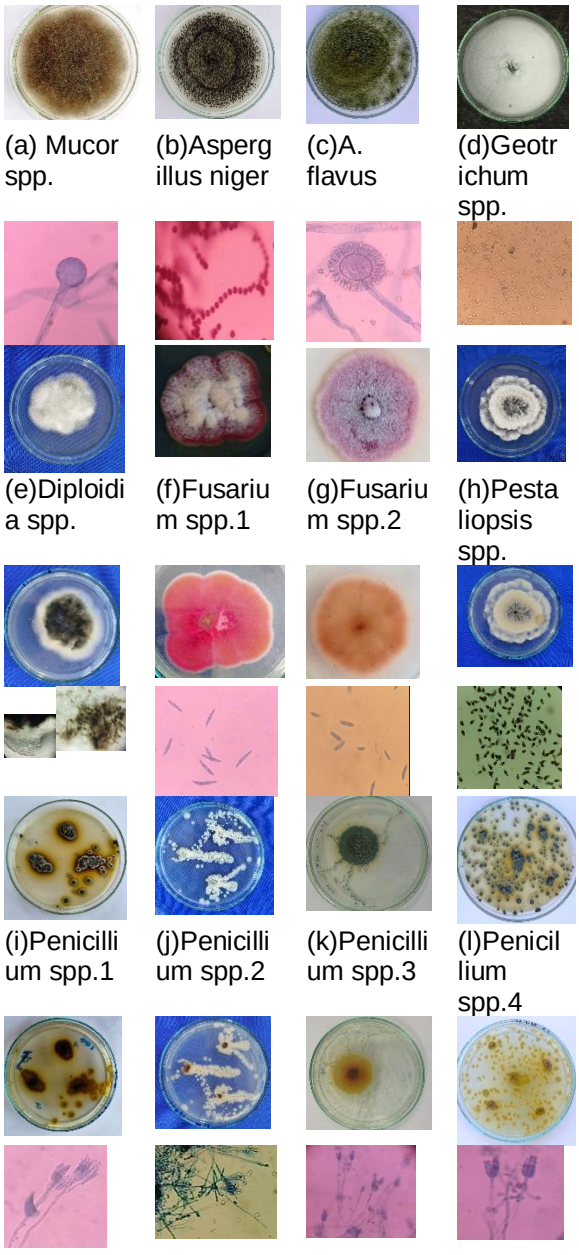


Table 2 : Frequency of occurrence of different post-harvest pathogens in Khasi mandarin of Meghalaya

Pathogen	Frequency of occurrence
Penicillium spp.	71.42
Aspergillus niger	42.85
Fusarium spp.	57.14
A. flavus	28.57
Mucor spp.	42.85
Geotrichum spp.	28.57

Plate 3 (a-l): Front, reverse and microscopical observation of isolated pathogens from the Khasi mandarin fruits



(a) Mucor spp. (b)Aspergillus niger (c)A. flavus (d)Geotrichum spp.

(e)Diploidia spp. (f)Fusarium spp.1 (g)Fusarium spp.2 (h)Pestaliopsis spp.

(i)Penicillium spp.1 (j)Penicillium spp.2 (k)Penicillium spp.3 (l)Penicillium spp.4

Table 3: Table of Cross-reference of cultural characterization of isolated post-harvest pathogens by the Royal Horticultural Society colour chart

Pathogen	Front view	Actual colour	Reverse view	Actual colour
Penicillium spp. 1	Green group 131 A	Winchester green cc 106	Yellow orange 22 A	Yellow ochre HCC 07
Penicillium spp. 2	Yellow green 147 A	Wood peeker green CC 244	Greyed orange 163 A	Bronge yellow CC 66
Penicillium spp. 3	Green group 137 A	-	Greyed orange 163 A	Bronge yellow CC 66
Penicillium spp. 4	Green group 139 A	Arras green CC 250	Greyed orange 170 C	-
Fusarium spp. 1	Red group 49 B	Venetian Pink HCC 420	Red group 49 B	Dawn Pink BCC 15
Diplodia spp.	Black group 202 A	Jet Black BCC 220	Black group 202 A	Jet Black CC 324
Fusarium spp. 2	Greyed Red group 181 A	-	Greyed Red group 181 A	
Geotrichum spp.	White Group B 155	-	Greyed yellow 160 D	-
Pestalotiopsis spp.	White Group 155 D	-	Greyed orange 163 C	-

3.1 COMPARISON OF DISEASE SEVERITY INDEX AMONG THE FOUR DIFFERENT INOCULATION METHODS

The first visible disease symptom was observed on the 3rd day of inoculation by disc inoculation method as water-soaked areas and within a few days the infected area enlarged with abundant sporulation zone surrounding a band of white mycelium. The infected area goes on increasing and ultimately the whole fruit is covered by the spores of the pathogen. Whereas, in the injection method, the first visible symptom was observed on the 5th day after inoculation as minute white dots in the injected areas, which progressed gradually and the area covered by sporulation of the inoculated pathogen. In the rest two inoculation methods, *viz.*, immersion and spraying, visible symptoms was observed on 6th and 8th day after inoculation. Disease severity was measured on 12th day after inoculation. Highest disease severity (Fig. 2) (83.33%) was recorded on the disc inoculation method (M4) followed by the injection method (M3) and the lowest was observed on spraying (M1) (25%). So, the method of spore application has a significant impact on the incidence of disease symptoms.

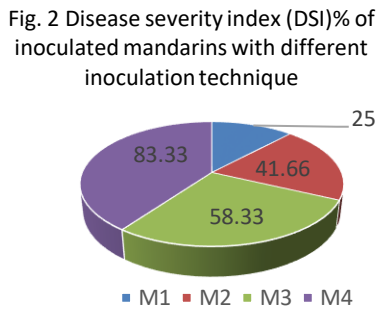


Fig. 2 Disease severity index (DSI)% of inoculated mandarins with different inoculation technique

Day	M1	M2	M3	M4

wise progress of diseases				
Day 1	-	-	-	-
Day 2	-	-	-	-
Day 3	-	-	-	+
Day 4	-	-	-	++
Day 5	-	-	+	++
Day 6	-	+	+	++
Day 7	-	++	+	++
Day 8	+	++	++	+++
Day 9	+	++	++	+++
Day 10	+	++	++	+++
Day 11	++	++	++	+++
Day 12	++	++	+++	+++

No disease= "-" , Mild diseases= "+", Moderate Disease= "++", Severe Disease="+++" (Barbedo *et al*., 2020)
M1: Spraying, M2: Immersion, M3: Injection, M4: Disc inoculation

Table 4: Observation on physical parameters of the inoculated fruit with four different inoculation methods

Days of observation	Physical parameters	M1: Spraying	M2: Immersion	M3: Injection	M4:Disc inoculation
1st day	Lustre	good	good	good	good
	Colour	orange	orange	orange	orange
	Shrinkage	No	No	No	No
2nd day	Lustre	good	good	good	good
	Colour	orange	orange	orange	orange
	Shrinkage	No	No	No	No
3rd day	Lustre	good	good	good	slight soft
	Colour	orange	orange	orange	pale yellow
	Shrinkage	No	No	No	slight
4th day	Lustre	good	good	good	soft
	Colour	orange	orange	orange	pale yellow
	Shrinkage	No	No	No	slight
5th day	Lustre	good	good	Soft	soft
	Colour	orange	orange	Pale Yellow	pale yellow
	Shrinkage	No	No	Yes	Yes

6th day	Lustre	good	Soft	Soft	soft
	Colour	orange	Pale Yellow	Pale Yellow	pale yellow
	Shrinkage	No	Yes	Yes	Yes
7th day	Lustre	good	Soft	Soft	soft
	Colour	orange	Pale Yellow	Pale Yellow	pale yellow
	Shrinkage	No	Yes	Yes	Yes
8th day	Lustre	Soft	Soft	Soft	very soft
	Colour	Pale Yellow	Pale Yellow	Pale Yellow	pale yellow
	Shrinkage	Yes	Yes	Yes	Yes
9th day	Lustre	Soft	Soft	Soft	very soft
	Colour	Pale Yellow	Pale Yellow	Pale Yellow	pale yellow
	Shrinkage	Yes	Yes	Yes	Yes
10th day	Lustre	Soft	Soft	Soft	very soft
	Colour	Pale Yellow	Pale Yellow	Pale Yellow	pale yellow
	Shrinkage	Yes	Yes	Yes	Yes
11th day	Lustre	Soft	Soft	Soft	very soft
	Colour	Pale Yellow	Pale Yellow	Pale Yellow	pale yellow
	Shrinkage	Yes	Yes	Yes	Yes
12th day	Lustre	Soft	Soft	Soft	very soft
	Colour	Pale Yellow	Pale Yellow	Pale Yellow	Pale yellow
	Shrinkage	Yes	Yes	Yes	Yes

Table 5: Day wise progress of the disease in four different inoculation methods

Visual observation of physical parameters:

Inoculated and uninoculated fruits were observed for three physical parameters like lustre, colour and shrinkages developed on different methods of inoculation from first day of inoculation till 12th day. It was observed that the tissues surrounding the inoculated disc were soften in the disc inoculation method from the third day onwards. Skin become pale coloured as well as shrinkages in the skin near the inoculated point was observed. On the other hand, in the injection and immersion method of inoculation, softening and pale yellow discoloration were noticed from the 5th day and 6th day onwards (Table 4 and 5). In the spraying method of inoculation, no symptom was observed until 7 days of inoculation. So, disc inoculation method is the fastest method for producing symptom while spraying method is the slowest method for producing disease symptoms on artificial inoculation.

3.2 PERCENT SEED INFECTION AMONGST FOUR DIFFERENT INOCULATION METHODS

Seed health is an important aspect. Though the orange seeds are usually thrown off, they are a rich source of calcium, potassium, phosphorus, oil, and antioxidants (Adubofuor *et al.,* 2021). Seeds are also used as propagating materials. In the current study, seed discolouration was observed in the inoculated mandarins. Percent seed infection was evaluated by the blotter test method (Plate 4 a-d) with four replications and found to be maximum in the disc inoculation method with a highest of 96%, followed by injection (88%)

and the lowest was recorded in the spraying method (76%) at 7 days after incubation (Fig 3). The discolouration and infection on the seed initially started from one side of the seed and gradually covered the entire area of the seed (Plate 5). Presterilized seeds with O.5% NaOCl isolated from *Penicillium* inoculated fruits by disc inoculation method, while plated on an agar plate, growth of profuse green to dark green mycelia from tip of the seed (Plate 6 a-b) or radicle was observed, which is the store house of nutrients and water required for translocation during seed germination.

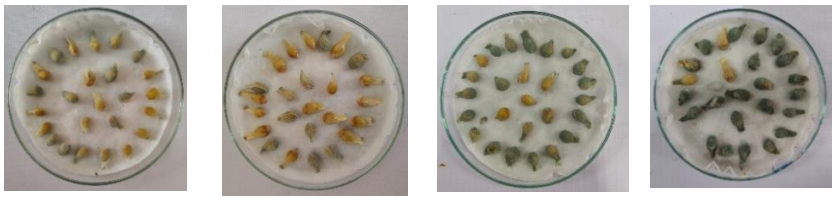


Plate 4 (a-d) Blotter test: M1: Spraying, M2: Immersion, M3: Injection, M4: Disc inoculation method respectively.

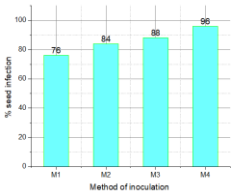


Fig 3. Percent seed infection in blotter test

Plate 5: Discoloured seed


Plate 6 (a-b) Growth of pathogenic mycelia from the seed of inoculated mandarins


Plate 7a: Pulp infection in the inoculated mandarins


7(b) 7(c)

7(b-c): Loose and intact pulp in inoculated (b) and uninoculated mandarins (c)

3.3 PULP INFECTION

Inoculated fruits were carefully peeled off after 12 days of incubation and each of the segments was observed for *Penicillium* infection**.** Growth and sporulation of *Penicillium* species in flavedo and albedo of inoculated fruits were also observed in the disc method of inoculation. Individual fruit segments were found to be more or less covered with greenish fungal mycelium (Plate 7 a-c). Almost all the inoculated fruits by different inoculation methods lose their turgidity and compactness as compared to the uninoculated control.

4. DISCUSSION

Post-harvest losses are the biggest concern in developing countries like India. Combined harvest and post-harvest losses of oranges range up to 25-30% (Doki *et al*., 2019). Fungal infection of the fruit may occur during the growing season, ripening, harvesting, processing, handling, and storage. High sugar and water content along with low pH make the fruit suitable for fungal attack (Singh and Sharma, 2007). Five different fungal genera were isolated from the Khasi mandarin fruit as associated pathogens for post-harvest decay and were identified as *Penicillium* spp., *Aspergillus* spp., *Mucor* spp., *Geotrichum* spp. and *Fusarium* spp. *Penicillium* spp. were widely occurring amongst the collected fruit

samples, followed by *Fusarium , Aspergillus* and *Mucor* spp. These findings are in line of Oviasogie *et al.,* 2015 who reported many post-harvest pathogens associated with *Citrus sinensis* of Benin city, Edo state, Nigeria. Tariang *et al.,* (2019) also reported *Penicillium* rot as a major constraint in Khasi mandarin production and storage from North East India. *Penicillium* ear rot of Maize kernels is one of the serious post-harvest diseases in the maize industry, where blue-green growth occurs on the cob surface. Similarly, Medic Pap *et al*., (2022) also reported 3-13.3% *Penicillium* infection on storage onion seeds. The growth of the *Penicillium* mycelium was observed from one side of the maize seed (Erasto *et al*., 2023), similar findings are also recorded in the present study. Application of sporulating mycelial disc to the injured mandarins can quickly develop lesions as compared to spraying of spore load to an uninjured one. The present investigation confirms that the *Penicillium* is a wound loving fungus. Which is in line of the work conducted by Kavanagh and wood (1967) they proved the role of wounded fruit on symptom development as compared to un injured one. The coloured flavedo of oranges is full of oil glands. While wounding, these oil glands release essential oils which kill the flavedo cells and colonization of dead flavedo cells helps to entry of the pathogens through the thin parenchymatous cell and reach the albedo, and sporulation starts in the fruit surface. Lesions on the skin develops on successful penetration of the albedo cells. The injury in the albedo layer, which is more spongy as well as having more intercellular spaces than the flavedo layer of the mandarin, makes it more susceptible to producing symptoms (Kavanagh and wood 1967). In the disc inoculation method, mycelial discs are in direct contact with the albedo, making the easier for proliferation of the pathogen and thereby more quickly symptom develop than the rest of the inoculation method. Moreover, on *Penicillium* inoculation, production of pectinases, cellulases, and polygalacturanases degrade the fruit cell wall, resulting shrinkages of the skin which was observed in the inoculated fruits (Papoutsis *et al.*, 2019). The infected epicarp or flavedo and whitish mesocarp or albedo undergo plasmolysis, resulting development of soft watery spots in the infection site (Han *et al*., 2013) followed by gradual development of whitish mycelia and greenish sporulation, ultimately fruit is rotten.

5. CONCLUSION

The present research emphasises the need for improved storage practices of Khasi mandarin (*Citrus reticulata*) which is a unique germplasm of North East India having the highest export potential. To conserve this germplasm and to increase its storability, the present research focused on the pathogen that are associated with the short shelf life of Khasi mandarin and also gives insights into the different methods of inoculation of pathogens into the fruit and the impact of inoculated *Penicillium* in the fruit as well as seed and pulp texture. Khasi mandarin, the king of oranges (Singh *et al*., 2022) is rich in biochemical composition, physicochemical properties, mainly its bright orange colour, thin peel, more sweeter juice content make it a unique germplasm of North East India. Currently, India is in the third position in the production of oranges. To raise the position or to keep in the same, the productivity and yield of the crop should be enhanced. Awareness programme must be created in the mandarin growers of North East India, specialty the Khasi tribe, to enhance its productivity as well as increase its storage life. Future research should be focused on increasing the storability either by use of bioagents like *Bacillus subtilis* (Tariang *et al*., 2019) or antimicrobial nanomaterials (Singh *et al.,* 2022) as a coating material with an eco-friendly approach that can effectively enhance the shelf life of Khasi mandarin.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal

ETHICAL DECLARATION

The submitted work is original and has not been published elsewhere in any form or in language. The collection of the plant parts used in the present study complied with local guidelines.

CONFLICTS OF INTEREST The authors declare there is no conflicts of interest.

DATA AVAILABILITY

The data supporting this study's findings are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at CPGS-AS, Umiam, Central Agricultural Institute (Imphal).

References:

Adubofuor, J., Akyereko, Y.G., Batsa, V, Apeku, O.D., Amoah, I. and Diako, C.. (2021). Nutrient composition and physical properties of two orange seed varieties. *International Journal of Food Science*. *11*: 6415620. https://doi.org/10.1155/2021/6415620

Annonymous (2014) Khasi Mandarin-GI application No. 465. Geographical Indications Journal No.63. Government Of India, 24-34.

Bock, C.H., Barbedo, J.G.A., Del, Ponte., E.M., Bohnenkamp, D. & Mahlein, A.K. (2020). From visual estimates to fully automated sensor-based measurements of plant

disease severity: status and challenges for improving accuracy. *Phytopathology Research 2*: 9. https://doi.org/10.1186/s42483-020-00049-8.

Doki, N., Eya, C. & Tughgba M. (2019). Determinants of post-harvest losses of orange in selected local government areas of Benue state. *International Journal of New Economics and Social Sciences* https://doi.org/10.5604/01.3001.0013.8106.

Erasto, R., Kilasi, N. & Madege, R.R. (2023). Prevalence and Management of Phytopathogenic Seed-Borne Fungi of Maize. Seeds, 2, 30-42. https://doi.org/10.3390/seeds2010003

Han, QZhao H., Cheng, Y., Yao, J., Huang, L. & Kang, Z. (2013). A cytological study on infection of citrus fruits by *Penicillium digitatum*. *Mycosystema*. *32*:967–977. (In Chinese)

Jkavanagh, J.A. &Wood, R.K.S. (1967). The role of wounds in the infection of oranges by Penicillium digitatum Sacc*. Annals of Applied Biology, 60*(3), 375–383*.* https://doi.org/10.1111/j.1744-7348.1967.tb04490.x

Kumar, N., Khan, A. A., Pyngrope, D., Alanzi, A.M., Upadhyay, A. & Shukla, S. (2024) Development and characterization of novel starch (mango kernel and litchi seed) based active edible coatings and films using ultrasonication Effects on postharvest shelf life of *Khasi* mandarins. *Sustainable Chemistry and Pharm*acy https://doi.org/10.1016/j.scp.2024.101610

Medic-Pap, S., Tančić Živanov, Sonja, D. D., Ignjatov, M. & Ilić, A. G., Svetlana, Gvozdanović-Varga, Jelica. (2022). Seedborne fungi on stored onion seeds. Zbornik Matice srpske za prirodne nauke. https://doi.org/10.2298/ZMSPN2243039M

Oviasogie, F.E., Ogofure, A.G., Beshiru, A., Ode, J.N. & Omeje, F.I. (2015). Assessment of fungal pathogens associated with orange spoilage*. African Journal of Microbiology Research*. *9* (29)1758-1763 DOI: 10.5897/AJMR2014.7246

Papoutsis, K., Mathioudakis, M. M., Hasperué, J.H. & Ziogas, V. (2019). Non chemical treatments for preventing the postharvest fungal rotting of citrus caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). *Trends in Food Science and Technology*. *86*, 479–491. http://dx.doi.org/10.1016/j.tifs.2019.02.053

Pitt, J.I. & Hocking, A.D. (1997). Fungi and food spoilage. Blackie Academic and Professional, an imprint of Chapman and Hall, Second Edition p.593.

Royal Horticultural Society Colour Chart, British Colour Council Dictionary of colour standards

Samson, R.A., Hoekstra, E.S. & Frisvad, J.C. (2004). Introduction of food-borne fungi. Seventh Edition. Central bureau voor Schimme lcultures P. 389.

Singh, D. & Sharma, R.R. (2007). Post-harvest diseases of fruit and vegetables and their management. In: Prasad, D. (Ed.), Sustainable Pest Management. Daya Publishing House, New Delhi, India.

Singh, Y. D., Das, D., Das, S., Swain, K. D., Pradhan, S. & Babu, P. J. (2022) Pharmacological activities of limonin from Khasi Mandarin as therapeutic applications, *Pharmacological Research - Modern Chinese Medicine*, 5,100181, ISSN 2667-1425, https://doi.org/10.1016/j.prmcm.2022.100181.

Tariang, J., Majumder, D. & Firake, D. M. (2019). Biocontrol Management of Postharvest *Penicillium* Rot Disease of Khasi Mandarin (*Citrus reticulata* Blanco) Oranges Using Native *B. subtilis* in Meghalaya, India *International Journal of Pure and Applied Bioscience. 7* (2): 292-302 (2019) http://dx.doi.org/10.18782/2320-7051.7186