

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

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15 **1.INTRODUCTION**

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

33 are responsible for short shelf life of the fruit. to diagnose the most potent post-harvest
34 pathogens associated with khasi mandarin and to evaluate the most efficient artificial
35 inoculation methods, that leads to *penicillium* rot, the present investigation was carried out in
36 2023-2024 and 2024-2025 at college of post graduate studies in agricultural sciences,
37 Umiam, Meghalaya, India

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39 **2.METHODOLOGY**

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41 **2.1 COLLECTION OF SAMPLES**

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

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49 **2.2 ISOLATION AND PURIFICATION OF PUTATIVE PATHOGENS**

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

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

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66 **2.3 PATHOGENICITY TEST**

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

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74 **2.4 INOCULATION OF DOMINATE PATHOGEN WITH FOUR DIFFERENT 75 INOCULATION METHODS**

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77 The most dominant *Penicillium* species confirmed from the pathogenicity test was
78 inoculated with four different inoculation methods viz., spraying, immersion injection and disc
79 inoculation method. Spore concentration of 2.65X10⁶ CFU/mL was used for spraying,
80 immersion as well as injection method. Freshly prepared spore suspension was sprayed on
81 the fully ripened equal size fruits with the help of hand held atomizer with the pore size of 0.8
82 mm till the run off condition and the fruits were kept in pre sterilized tray. For immersion
83 method of inoculation, fruits were dipped in 250 ml conidial suspension with the above
84 mentioned spore concentration for a period of 1 minutes and air dried the inoculated sample

85 and kept in sterilized tray. For injection method of inoculation, spore suspension were
 86 directly inserted into the skin of the sterilized fruits in four equidistant position and injected
 87 till run off condition. Inoculated mandarins were kept in pre sterilized tray. For disc
 88 inoculation, 6 mm disc from freshly prepared culture plates were inoculated with the help of
 89 a sterile cork borer in four equidistant positions of Khasi mandarin fruits. The inoculated fruits
 90 were kept in a pre-sterilized plastic tray and all the trays were covered with a perforated
 91 transparent polythene sheet and incubated at room temperature ($28\pm 1^{\circ}\text{C}$). The fruits were
 92 observed each day for the appearance of visible symptoms.

94 2.5 ESTIMATION OF DISEASE SEVERITY INDEX (DSI)

95
 96 The per cent disease severity index was calculated with the following disease rating
 97 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.

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

$$101 \text{ DSI}(\%) = \frac{\sum(\text{No. of diseased fruit in each rating category} \times \text{Severity rating})}{\text{Total No. of fruit assessed} \times \text{Highest Scale}} \times 100$$

102
 103 The inoculated mandarins were also observed for pulp and seed infection, if any to
 104 investigate whether the infection was moved to the pulp and seed. Each of the segments
 105 from the inoculated mandarins was observed for *Penicillium* infection by removing the outer
 106 peel and evaluating the condition of each segment of the fruit by observing its compactness,
 107 colour and texture.

109 2.6 ESTIMATION OF PERCENT SEED INFECTION (%)

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

$$119 \text{ Percent seed infection} (\%) = \left(\frac{\text{Number of infected seed}}{\text{Total number of seed per plate}} \right) \times 100$$

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

$$122 \text{ Weight loss}(\%) = \left(\frac{W_i - W_s}{W_i} \right) \times 100$$

123 Where,

124 W_i = Initial fruit weight

125 W_s = Fruit weight at sampling date

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127 **3. RESULTS AND DISCUSSION**

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

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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 discoloration and white mycellial growth.



(f) Blueish green mycellial growth with blackish discoloration.



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



(h) White irregular mycellial growth with brownish discoloration.

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

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2(a)Fusarium spp.



2(b)Pestalotia spp.



2(c)Penicillium spp.



2(d)Penicillium spp.



2(e)Penicillium spp.

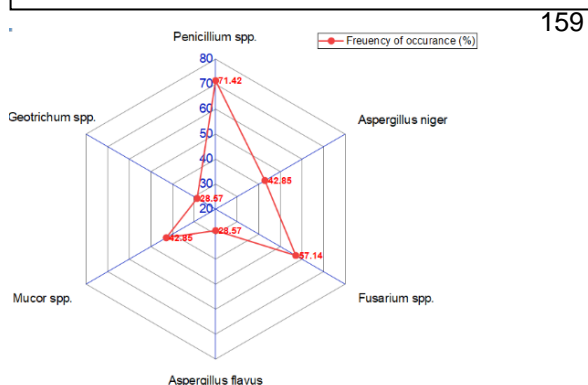


2(f)Penicillium spp.

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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



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

168 Table 2 : Frequency of occurrence of
 169 different post-harvest pathogens in Khasi
 170 mandarin of Meghalaya
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Pathogen	Frequency of occurrence
<i>Penicillium</i> spp.	71.42
<i>Aspergillus niger</i>	42.85
<i>Fusarium</i> spp.	57.14
<i>A. flavus</i>	28.57
<i>Mucor</i> spp.	42.85
<i>Geotrichum</i> spp.	28.57

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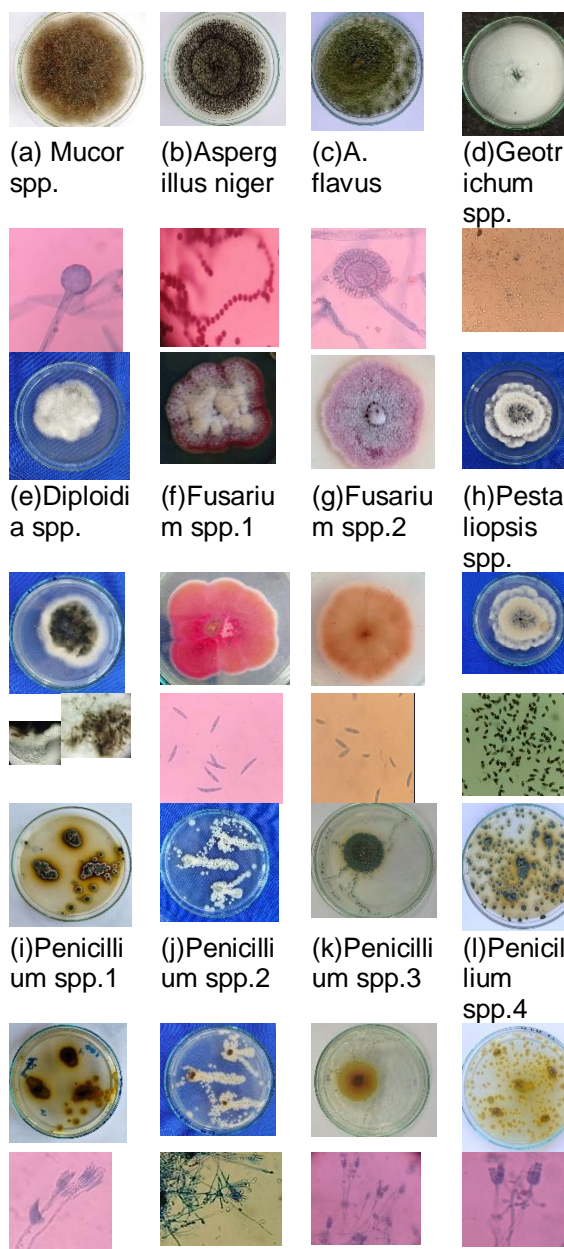


Table 3: Table of Cross-reference of cultural characterization of isolated post-harvest pathogens by the Royal Horticultural Society colour chart

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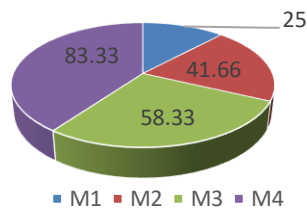
Pathogen	Front view	Actual colour	Reverse view	Actual colour
Penicillium spp. 1	Green group 131 A	Winchester green cc 106	Yellow orange 22 A	Yellow ochre HCC 07
Penicillium spp. 2	Yellow green 147 A	Wood pecker green CC 244	Greyed orange 163 A	Bronge yellow CC 66
Penicillium spp. 3	Green group 137 A	-	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	-

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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

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



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lowest was observed on spraying (M1) (25%). So, the method of spore application has a significant impact on the incidence of disease symptoms.

Day	M1	M2	M3	M4
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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

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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

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

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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

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

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Visual observation of physical parameters:

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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.

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3.2 PERCENT SEED INFECTION AMONGST FOUR DIFFERENT INOCULATION METHODS

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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 discoloration 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%)

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263 and the lowest was recorded in the spraying method (76%) at 7 days after incubation (Fig 3).
 264 The discolouration and infection on the seed initially started from one side of the seed and
 265 gradually covered the entire area of the seed (Plate 5). Presterilized seeds with 0.5% NaOCl
 266 isolated from *Penicillium* inoculated fruits by disc inoculation method, while plated on an agar
 267 plate, growth of profuse green to dark green mycelia from tip of the seed (Plate 6 a-b) or
 268 radicle was observed, which is the store house of nutrients and water required for
 269 translocation during seed germination.
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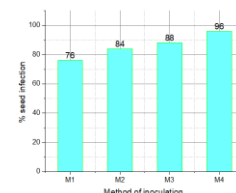
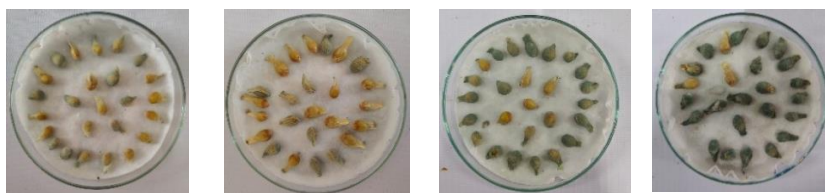


Plate 4 (a-d) Blotter test: M1: Spraying, M2: Immersion, M3: Injection, M4: Disc inoculation method respectively.

Fig 3. Percent seed infection in blotter test

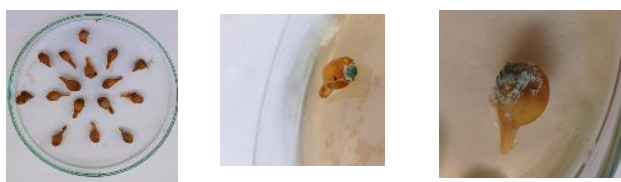


Plate 5: Discoloured seed

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

271 **3.3 PULP INFECTION**

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

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 281 Post-harvest losses are the biggest concern in developing countries like India.
 282 Combined harvest and post-harvest losses of oranges range up to 25-30% (Doki *et al.*,
 283 2019). Fungal infection of the fruit may occur during the growing season, ripening,
 284 harvesting, processing, handling, and storage. High sugar and water content along with low
 285 pH make the fruit suitable for fungal attack (Singh and Sharma, 2007). Five different fungal
 286 genera were isolated from the Khasi mandarin fruit as associated pathogens for post-harvest
 287 decay and were identified as *Penicillium* spp., *Aspergillus* spp., *Mucor* spp., *Geotrichum* spp.
 288 and *Fusarium* spp. *Penicillium* spp. were widely occurring amongst the collected fruit

289 samples, followed by *Fusarium*, *Aspergillus* and *Mucor* spp. These findings are in line of
290 Oviasogie *et al.*, 2015 who reported many post-harvest pathogens associated with *Citrus*
291 *sinensis* of Benin city, Edo state, Nigeria. Tariang *et al.*, (2019) also reported *Penicillium* rot
292 as a major constraint in Khasi mandarin production and storage from North East India.
293 *Penicillium* ear rot of Maize kernels is one of the serious post-harvest diseases in the maize
294 industry, where blue-green growth occurs on the cob surface. Similarly, Medic Pap *et al.*,
295 (2022) also reported 3-13.3% *Penicillium* infection on storage onion seeds. The growth of
296 the *Penicillium* mycelium was observed from one side of the maize seed (Erasto *et al.*,
297 2023), similar findings are also recorded in the present study. Application of sporulating
298 mycelial disc to the injured mandarins can quickly develop lesions as compared to spraying
299 of spore load to an uninjured one. The present investigation confirms that the *Penicillium* is a
300 wound loving fungus. Which is in line of the work conducted by Kavanagh and wood (1967)
301 they proved the role of wounded fruit on symptom development as compared to an injured
302 one. The coloured flavedo of oranges is full of oil glands. While wounding, these oil glands
303 release essential oils which kill the flavedo cells and colonization of dead flavedo cells helps
304 to entry of the pathogens through the thin parenchymatous cell and reach the albedo, and
305 sporulation starts in the fruit surface. Lesions on the skin develops on successful penetration
306 of the albedo cells. The injury in the albedo layer, which is more spongy as well as having
307 more intercellular spaces than the flavedo layer of the mandarin, makes it more susceptible
308 to producing symptoms (Kavanagh and wood 1967). In the disc inoculation method, mycelial
309 discs are in direct contact with the albedo, making the easier for proliferation of the pathogen
310 and thereby more quickly symptom develop than the rest of the inoculation method.
311 Moreover, on *Penicillium* inoculation, production of pectinases, cellulases, and
312 polygalacturanases degrade the fruit cell wall, resulting shrinkages of the skin which was
313 observed in the inoculated fruits (Papoutsis *et al.*, 2019). The infected epicarp or flavedo and
314 whitish mesocarp or albedo undergo plasmolysis, resulting development of soft watery spots
315 in the infection site (Han *et al.*, 2013) followed by gradual development of whitish mycelia
316 and greenish sporulation, ultimately fruit is rotten.

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318 5. CONCLUSION

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

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358 CONSENT

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

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365 ETHICAL DECLARATION

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

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

372

373 DATA AVAILABILITY

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

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