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PATULIN AND CITRININ CORRELATE WITH POST-HARVEST FRUIT ROT OF PEAR BY *PENICILLIUM EXPANSUM*

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ABSTRACT

The plant pathogenic fungus *Penicillium expansum* consistent production of mycotoxins viz. patulin and citrinin were detected to be correlated with *Penicillium expansum* rotted pear fruits. Both toxins were observed in very high amounts. Patulin was detected up to 36mg/kg whereas citrinin level was comparatively decreases up to 6.04mg/kg of the pears. These toxin amounts are immoderate in comparison to WHO recommended tolerance level. Present research work, therefore, suggested that proper storage and grading of pears before marketing is mandatory, so that *Penicillium expansum* rotted fruits are not consumed.

KEYWORDS: Pears, Mycotoxin, Citrinin, Patulin, Post-harvest.

INTRODUCTION

Pear is an important fresh rosaceous fruit grown in the temperate areas of Jammu and Kashmir. It is juicy, astringent, nutritionally rich in sugars, organic acids, vitamins, Phosphorous, iron and is valued mainly as a dessert fruit. Before harvesting, they are attacked by a few fungi[3] but after harvesting their susceptibility to fungal pathogens increases.[16] During a survey conducted at APMC fruit market Vashi, Navi Mumbai, pears were found to suffer extensive post-harvest blue mold rot caused by *Penicillium expansum*. In view of this, an attempt was made to evaluate the natural occurrence of *Penicillium* toxins associated with this fruit during pathogenesis.

MATERIALS AND METHODS

Isolation of *Penicillium expansum* isolates from rotted pears

Pear suffering from blue mold rot was collected in sterilized bags at regular intervals from

wholesaler and retailer. Isolation was made within 24 hours of their collection by streaking the spore on sterilized potato dextrose ager medium.

Preparation of spore suspension

Spore suspension of *P. expansum* isolates was prepared by flooding 4 days old culture grown on slant with 10ml of sterile distilled water containing 0.1% Tween 20. Spores were rubbed from surface of the slant with a glass rod, passed through two layers of sterilized cheesecloth, counted with a haemocytometer and adjusted with sterile distilled water to obtain 10^5 Spores/ml.

Inoculation of pear fruits

Mature and healthy pear fruits were initially sterilized by dipping in 95% ethanol for 30 min., rinsed with sterilized water and then dried under sterilized conditions. Therefore, single wound 4mm wide and 10mm deep was made in the fruit with the help of a sterilized cork borer and 50µl of spore suspension was inserted into the wound through a sterilized micropipette. Inoculated fruits were incubated at 28 ± 2°C and 100% relative humidity for 15 days.

Extraction and estimation of patulin and citrinin from diseased fruit

Natural production of patulin and citrinin mycotoxin was estimated at the end of incubation period by taking 30gm of fruit pulps adjacent to the inoculated region and extracting it by the method of Gimeno and Martins, (1983). Chloroform extract thus obtained were collected and evaporated to 0.5ml. This concentrated extract was dissolved to make total volume of 1ml for thin layer chromatography. Quantitative separation of these toxins performed on activated TLC plates using Tolune: Ethylacetate: Chloroform (80:70:50) and 1ml of 90% formic acid. For detection of patulin, developed plates were sprayed with freshly prepared phenylhydrazine hydrochloride and the heated for 5 minutes at 110°C.[15] Under visible light, patulin appeared yellow spots. For the detection of citrinin, developed plates were directly visualized under 365nm UV light and they showed yellow fluorescent spots. Standard patulin and citrinin samples were also spotted on the TLC plates as reference spots. Further chemical confirmation of citrinin was done by spraying aluminium chloride on the developed plate. Heated it for 5 minutes at 110°C, cooled and observed under 365 nm UV light. This chemical test changed the yellow fluorescent of citrinin to sky blue fluorescent.[5]

For quantitative estimation of patulin and citrinin, spectophotometric method of Bacha *et. al.*, (1988) was followed. For patulin estimation, silica gel of each yellow spot was scraped in a clean dry centrifuge tube, dissolved in 5ml of n-butanol and centrifuged at 3000rpm for 3min. Citrinin estimation was done by marking yellow fluorescent spots under long wave UV light (365 nm) scraped them individually in clean, dry centrifuge tubes, dissolved them in 5ml of cold methnol and centrifuged at 3000rpm for 3 minutes. Colour intensity of supernatant was immediately determined in a spectrophotometer at 540nm for patulin and at 332nm for citrinin.

RESULTS AND DISCUSSION

Results are depicted in **Table 1** showed that 86.7% of the recovered isolates were found to be toxigenic producing either patulin or citrinin or both. Among these, positive 33.3% were found to be patulin producers (28 to 36 mg/kg) whereas, 80.0 % of the tested *Penicillium expansum* isolates were found to be positive for citrinin production (0.09 to 6.04 mg/kg). Similar results were acquired by Brian *et. al.*, (1956). The production of patulin in fruits may be attributed to the sugar, organic acid.[12]

From the result it was also detected that 26.7% isolates of *Penicillium expansum* could produce both patulin and citrinin and their levels of production were very high. Correspondingly, amount of patulin and citrinin contamination from *Penicillium expansum* infected pears has been stated to be as high as 1 and 3 mg/kg.[5, 14] Although there is presently no confirmation to prove that patulin and citrinin have the potential to produce disagreeable human health effects.[2,10] In addition the finding in animals that these mycotoxins are carcinogenic, teratogenic and nephrotoxic[9,4,11] emphasize the need for analyze. World health Organization (WHO) has advocated a maximal patulin level of 50µg/kg. Various European and other countries have also suggested highest patulin levels of 30-50 µg/kg.[8] But even now, no recommendation for the tolerance level of citrinin has been made. Since patulin is a stable mycotoxin[13,6], and its co-occurrence with citrinin in the infected pears and the products made from pears rotted by *Penicillium expansum*, which may present a health hazard.

This evaluation, therefore, shows that proper storage and sorting of pears is very necessary before marketing so that intake of these mycotoxins above the tolerance limit is avoided. This will essentially make a difference for children and vegetarians who consume a higher quantity of fruits.

Table 1: Production of patulin and citrinin in *Penicillium expansum* infected pears.

	Total no. of isolates screened= 15.0	
	% of toxigenic isolates	= 86.7
Isolates screened	% of patulin producer	= 33.3
	% of citrinin producer	= 80.0
	% of both patulin and citrinin Producer	= 26.7
	Patulin toxin	Citrinin toxin
Pe-1	-	6.04 ± 7.1
Pe-2	29.50±6.5*	0.94 ± 2.0
Pe-3	-	0.26 ± 1.7
Pe-4	-	0.09 ± 2.4
Pe-5	-	0.72 ± 1.8
Pe-6	-	0.15 ± 3.4
Pe-7	-	4.21 ± 2.4
Pe-8	-	0.39 ± 1.8
Pe-9	-	-
Pe-10	28.00±3.5	1.15 ± 6.4
Pe-11	33.50±7.5	-
Pe-12	-	-
Pe-13	36.00±5.8	0.14 ± 2.4
Pe-14	-	0.18 ± 1.4
Pe-15	33.50±8.5	2.00 ± 1.6

*Each value represents the mean ± SD, n = 3, and - not detected.

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