

Original Research Article

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Effect of Inoculum Load on Disease Initiation, Threshold Point and Population Build Up of Post Harvest Pathogen (*Penicillium islandicum* Sopp.) of Indian Gooseberry (*Emblica officinalis* Goerth)

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ABSTRACT

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Studies were undertaken to find out the effect of inoculum load of post harvest pathogen (*Penicillium islandicum*) on disease initiation, threshold point and population build up of blue mould rot in Indian gooseberry. Inoculations were made on the fruits of Indian gooseberry at five concentrations (10, 50, 100, 200 and 500 spores/ml.). The disease initiation commenced after 54 hrs. of inoculation with inoculum load of 500 spores/ml followed by 200 spores/ml where the initiation of disease was observed after 72 hrs. At lower spore load (10 spores/ml), there was no disease initiation even after 108 hrs of inoculation. Minimum number of spores required to initiate infection after 102 hrs was 50 spores/ml which can be considered as the threshold point. Maximum population build up (2×10^6 and 2×10^8) of the pathogen occurred at 500 spores/ml after 5 and 10 days of inoculation, respectively.

Introduction

Aonla or Indian gooseberry (*Emblica officinalis* Goerth. Syn. *Phyllanthus emblica* L.) belongs to family *Euphorbiaceae* order *Euphorbiales* is one of the most important indigenous fruit of Indian sub continent. It has been cultivated in India since time immemorial. It is grown in tropical and subtropical parts of India, China, Indonesia and the Malay Peninsula (Golechha *et al.*, 2012 and Srivasuki, 2012). In India, nearly 20-

35 per cent of perishables fruits and vegetables are lost due to post harvest diseases (Rawal and Saxena, 2005). Among post harvest diseases of aonla, fruit rot caused by *Penicillium islandicum* Sopp. is the most important as it affects the fruit quality and quantity in relation to the market value. The open wounds, created during harvesting, handling and packaging are the major sites of invasion by post harvest wound pathogens. The major loss due to blue mould rot in aonla takes place from transit to the market. The

fruits falling to the ground also succumb to infection. The earliest symptoms of infection due to *Penicillium islandicum* is seen as water soaked lesion on the fruit surface which enlarges in size followed by development of pin head size colonies of golden yellow colour. The older colonies turn olive green.

In a study, it was found that 108 conidia ml⁻¹ of *A. alternata* in tomato resulted in 93.4 per cent infection recorded after 7 days of incubation meaning there by that 108 conidia ml⁻¹ could serve as the inoculum potential of *A. alternata* (Verma, 2004). Yousefi and Hagian Shahri (2009) conducted pathogenicity tests by inoculating slightly wounded plant tissue with conidial suspension adjusted to 1.5×10⁴ conidia ml⁻¹ of *Alternaria alternata* using a haemocytometer that was capable of causing infection within 2-4 days. *Alternaria* isolates was capable of causing infection on citrus leaves from suspension of conidia (105 conidia /ml) prepared from 5-7 days old PDA cultivated isolates (Kakvan *et al.*, 2012).

Barring a few reports about its occurrence, hence, comprehensive studies have not been under taken regarding this pathogen of aonla fruit, taking into consideration the medicinal value of the fruit, economic losses by the disease and inadequate information, the investigation was carried out to find out the effect of inoculum load of post harvest pathogen (*Penicillium islandicum*) on disease initiation, threshold point and population build up of blue mould rot in Indian gooseberry.

Materials and Methods

The study on disease initiation, threshold point and population build up at optimum temperature and humidity was conducted. Spore suspension, from seven days cultures, prepared in distilled and sterilized water and the initial concentration was adjusted to 1000 spores/ml with the help of haemocytometer.

Serial dilutions were made to obtain 1000, 500, 200, 100, 50 and 10 spores/ml with sterile distilled water. Fruits were inoculated using 0.5 ml of test spore suspension and punctures were plugged back. The injury was sealed with sterile paraffin wax and incubated at 27 ± 1⁰C under aseptic conditions. Inoculated fruits were examined after every 6 hours for disease initiation and threshold point of the pathogen. Subsequent build up of population was assessed after 10 days of incubation. The diseased portion was scooped out with knife and the percentage rot was calculated. One gm of diseased tissue was homogenized in 5 ml of water with a waring blender and the concentration of the spores was recorded with the help of haemocytometer.

Results and Discussion

Effect of inoculum load on disease initiation and threshold point

In order to study the effect of varying inoculum load on the establishment of the disease, aonla fruits were injured and inoculated with 0.5 ml spore suspension of different concentrations ranging from 5 to 100 spores/ ml. Inoculated fruits were incubated at 27 ± 1⁰C and observations were recorded at 6 hours interval up to 108 hours for initial appearance of visible symptoms. The data presented in Table 1 indicated that the time required for appearance of visible symptoms decreased with the increase in amount of inoculum. Concentration of 5 spores/ml failed to cause infection indicating that the threshold point for the infection is more than 5 spores/ml. No symptoms were visible up to 84 hours with a spore load of 50 spores/ml and symptoms could be noticed only after 90 hours. The disease was visualized within 48 hours when 250 spores were used. The rate of development was also faster with increased inoculum load.

Effect of inoculum load on population build up

The inoculum load has influenced the disease development as well as the rate of multiplication of the fungus as observed from the number of spores produced from variously inoculated fruits. Perusal of data from Table 2 indicated that the number of propagules produced increased proportionately with the number of spores used for inoculating the fruits. The number of spores produced on the fruits varied from 1.9×10^5 to 2.1×10^8 for 25 to 250 spores/ml inoculum.

Average of four replications

Investigations on the inoculum potential of *Penicillium islandicum* on aonla at optimum temperature and humidity has revealed that the time required for the establishment of infection is positively correlated with the inoculum load. The subsequent rate was also

faster with the increasing amount of inoculum. This observation is in agreement with the earlier reports of Spotts and Cervantes (2001) and Luo *et al.*, (2001). The rate of multiplication has shown positive correlation with amount of inoculum. A minimum number of 100 spores/ml was necessary for initiating the disease which indicated that threshold point for this fungus is 50 spores per inoculation. On the contrary, Noe and Starkey (1982) did not record bitter rot infection in apple at an inoculum of even 10^3 conidia per ml which may be due to different host parasite relationship.

A minimum number of 50 spores/ml was necessary for disease initiation which indicates that inoculum threshold point of this fungus to cause infection is 25 spores/inoculation. Maximum population build up of *Penicillium islandicum* was observed at the concentration of 500 spores/ml after 5 and 10 days of inoculation on the fruits.

Table.1 Effect of inoculum load on disease initiation and threshold point of aonla blue mould rot

Concentration of spores/ml	No. of spores / fruit (0.5 ml)	Disease initiation after hrs of inoculation										
		48	54	60	66	72	78	84	90	96	102	108
10	5	-*	-	-	-	-	-	-	-	-	-	-
50	25	-	-	-	-	-	-	-	-	-	±	+
100	50	-	-	-	-	-	-	±	+	++	+++	++++
200	100	-	-	-	±	+	++	+++	++++	Area of infection increasing		
500	250	±	+	++	+++	++++	Area of infection increasing					
	-	No infection										
	±	Suspected infection										
	+	Slight infection 5 mm diameter										
	++	10 mm diameter										
	+++	15 mm diameter										
	++++	20 mm diameter										

Table.2 Effect of inoculum load on population build up of *Penicillium islandicum* causing blue mould rot in aonla

Concentration of spores/ml	No. of spores / fruit (0.5 ml)	Population build up	
		After 5 –Days	After 10 –Days
10	5	-	-
50	25	1.9×10 ⁵ *	1.1×10 ⁷
100	50	4.8×10 ⁵	2.7×10 ⁷
200	100	1.2×10 ⁶	1.5×10 ⁸
500	250	1.8×10 ⁶	2.1×10 ⁸

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