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Impact of inoculation methods and fruit maturity on the development of *Aspergillus* fruit rot of citrus

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Abstract
Among the different methods of inoculation, Cork-wounding method (52.00%) found best for the Aspergillus rot development after 7th days of inoculation and it was at par with Pin-prick method (47.25 %). The fruits without injury failed to develop the rot. It was revealed that injury to fruit surface is essential for infection and development of Aspergillus rot in citrus. All the three stages of fruit maturity exhibited symptoms of the Aspergillus rot. However, highest Aspergillus rot severity was recorded in mature fruits (40.00 %) at 7th days after inoculation. Further it was noted that mature fruits are more prone to infection than the semi-mature and immature fruits.

Keywords: Aspergillus rot, citrus, inoculation methods, fruit maturity

Introduction
Citrus is an important fruit crop in the world and is cultivated in more than 100 countries covering all six continents, and it is often regarded as golden fruit or queen of all fruits (Nito, 1996). It is also known as Kagzi-lime, Acid lime, Sour lime, Mexican lime and in Hindi as "Neebu" and in Gujarati as "Leembu". Acid lime plant is a perennial profusely branched thorny shrub or small tree which bears more or less round oval, smooth fruits having thin rind attached lightly. Mature fruits of lime are light yellow in colour (Singh, 1995). Lime is a potential source of vitamin – C and it also extensively used for medicine and culinary purposes (Singh, 1995). The most popular citrus products prepared in India are squash, juice cordial, marmalade, pickles as well as essential oils from citrus peels is used for flavouring and perfumery trade, also in manufacturing of soap and resins etc. (Randhawa and Srivastava, 1986). Kaur and Verma (2002) observed a soft rot of citrus caused by *Aspergillus niger* up to 20 per cent in the orchard as well as in Punjab markets. A perusal of the available literature revealed that a very meager research work has been carried out on Aspergillus fruit rot (*Aspergillus niger*) of citrus.

Materials and Methods
Impact of inoculation methods on the development of Aspergillus fruit rot of citrus.
Healthy, fresh, matured, uniform size fruits of citrus were collected from the Horticulture Farm, AAU, Anand and brought to the laboratory in paper bags. The fruits were washed with tap water, then surface sterilized by dipping in 0.1 per cent $HgCl_2$ solution for one minute followed by three washings with distilled sterile water. The experiment was conducted in completely randomized design (CRD) with four replications. The fruits were injured by the following methods.

a. Stem end
The pedicel of fruits was completely removed from the stem end and fruits were injured at stem end with sterilized pins fixed on cork and then dipped in spore suspension (10^6 spores/ml) of the pathogen.

b. Stylar end
The fruits were injured at stylar end with sterilized pins fixed on cork and dipped in spore suspension (10^6 spores/ml) of test pathogen.

c. Rubbing
The fruits were rubbed with each other by shaking manually for five minutes in bamboo basket and then they were dipped in spore suspension (10^6 spores/ml) of test pathogen.

d. Pin prick
The fruits were injured by pin pricking on epicarp of fruit upto 2 mm depth with the help of sterilized pins fixed on cork. The fruits were then dipped in spore suspension (10^6 spores/ml) of test pathogen.

e. Cork Wounding
A hole of 2 mm diameter with 2 mm depth was made with the help of a sterilized cork borer on fruits, then the fruits were dipped in spore suspension of pathogen (10^6 spores/ml) and the hole was sealed with the bored tissues.

f. Fall Injury
Well matured healthy fruits were plucked from the tree and dropped to the ground from a height of 3 m. The fallen injured fruits were dipped in spore suspension (10^6 spores/ml) of test pathogen.

g. Without Injury (Control)
The fruits without any sort of injury were dipped in spore suspension (10^6 spores/ml) of test pathogen.
The inoculated fruits were placed in sterilized polythene bags. One fruit was accommodated in one bag. A piece of sterilized moist absorbent cotton swab was placed inside the bags and the mouths of the bags were loosely tied. The bagged fruits were incubated at 30 ± 1^0C. The observations on disease severity and incidence were recorded on 4th and 7th day of inoculation.

$$\text{Incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \text{ x } 100$$

The disease severity of rot was recorded on the basis of per cent infection by following assessment key.

Impact of fruit maturity on the development of Aspergillus fruit rot of citrus.
The fresh fruits of the desired stages of maturity were selected using the following key.

1. Immature fruits
Freshly harvested healthy, green and hard fruits.

2. Semi-mature fruits
Freshly harvested healthy fruits with greenish light yellow colour.

3. Mature fruits
Freshly harvested healthy fruits with yellow colour.
The healthy fruits of citrus were surface sterilized by dipping in 0.1 per cent $HgCl_2$ solution for one minute, followed by three washing with distilled sterile water. The fruits were injured by pin pricking on epicarp of fruit upto 2 mm depth with the help of sterilized pins fixed on cork. The fruits were then dipped in spore suspension (10^6 spores/ml) of test pathogen.

Results and Discussions
Influence of inoculation methods on the development of Aspergillus fruit rot on citrus
The data presented in Table 1 revealed that significantly highest Aspergillus fruit rot severity was recorded in Cork-wounding method (21.75 & 52.00%) after 4th and 7th days of inoculation over other methods, but it was at par with Pin - prick method (16.00 & 47.25 %). Cork - wounding method was found superior over rest of the methods except Pin - prick method. While rubbing method (6.00 & 13.25%) was found least effective. Aspergillus fruit rot symptoms were not developed in fruits inoculated without any injury. The results clearly indicated that the injury to fruit surface is essential for infection and further development of the rot. Rubbing, Pin-pricking, Cork-wounding and fall injury methods showed 100 per cent disease incidence, while Stem-end and Stylar-end methods exhibited 50 per cent disease incidence.
The results of present investigation corroborate with the results reported by Lal *et al*. (1981 & 1982) [2]. They showed injury is essential for the development of soft rot in ber (*Phomopsis natsume*) and aonla (*Phomopsis phyllanthi*). Cork-wounding and fall injury methods caused maximum rotting in aonla fruits caused due to *Penicillium islandicum*, *Colletotrichum gloeosporioides* and *Alternaria alternata* (Rathod, 2004) [5]. The maximum fruit rot severity was observed due to *Aspergillus niger* in Cork-wounding method (62.50 %) followed by Pin-prick method (43.87%) after 10th day of inoculation in aonla fruits (Nath, 2006) [3]. Similar trend of results was reported by Yadav (2000) [6] indicating Cork – wounding and Pin-pricking methods were most suitable for infection by *A. niger* in aonla.

Impact of fruit maturity on the development of Aspergillus fruit rot of citrus
In order to find out the most vulnerable stage of fruit during the process of maturity for infection and development of Aspergillus rot in citrus fruits, three stages of fruits were selected i.e. mature, semi-mature and immature. The results obtained are presented in Table 2. Significantly highest Aspergillus rot severity was observed in mature fruits (18.75 & 40.00%) as compared to semi-mature (12.50 & 33.25%) and immature (9.50 & 23.00%) fruits after 4th and 7th day of inoculation, respectively. Minimum rot severity was recorded in immature fruits (9.50 & 23.00 %) on 4th and 7th days of inoculation, respectively. It is clear from the results that as the fruit reaches towards maturity, it become more prone for infection and further development of Aspergillus rot (*A. niger*) of citrus.
Results of present findings are confirmative with the results obtained by Lal *et al*. (1982) [2] indicating that *Phomopsis phyllanthi* caused infection in young and mature aonla fruits, when inoculated artificially. The mature fruits were found more vulnerable for infection than the young ones. Ripening of fruit increased the susceptibility to rots caused by several fungi (Pathak, 1980). Yadav (2000) [6] observed higher Aspergillus rot severity in ripe fruits of aonla (11.25 & 20.00%) as compared to semi-ripe (10.00 & 17.50%) and unripe (7.25 & 13.25%) fruits after 3 and 6 day of inoculation.

Table 1: Influence of inoculation methods on development of Aspergillus fruit rot in citrus

S. No	Inoculation Methods	Aspergillus rot severity (%)		Disease incidence (%)
		4th day*	7th day**	7th day
1	Stem-end	14.00 (3.79)	27.76 (21.75)	50
2	Stylar-end	12.25 (3.56)	29.80 (24.75)	50
3	Rubbing	6.00 (2.54)	21.35 (13.25)	100
4	Pin-pricking	16.00 (4.34)	43.42 (47.25)	100
5	Cork-wounding	21.75 (4.70)	46.15 (52.00)	100
6	Fall injury	8.50 (2.99)	23.34 (15.75)	100
7	Control (Without injury)	0.00 (0.70)	0.00 (0.05)	0
S.Em. ±		0.139	1.703	---
C.D. at 5 %		0.408	5.011	---
C.V. %		8.58	6.82	---

* Figures in parentheses are square root transformed values, while outside are the original value.
** Figures in parenthesis are original value, while outside are the arc sine transformed value

Table 2: Impact of fruit maturity on the development of Aspergillus rot of citrus

S. No	Fruit maturity stage	Aspergillus rot severity (%)	
		4th day	7th day
1	Immature	9.50	23.00
2	Semi-mature	12.50	33.25
3	Mature	18.75	40.00
S.Em. ±		0.540	1.588
C.D. at 5 %		1.589	4.670
C.V. %		11.32	14.76

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