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Postharvest pathogenic behaviour of different litchi varieties

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Abstract
Litchi is a fruit grown in few parts of the world of subtropical climate. In India, it is a short duration crop in limited areas. The crop, due to its good marketable value, which depends on the negligible signs on fruit play an important role in the economy of farmers in the northern part of the country. The crop faces various abiotic and biotic obstacles in a healthy production. Among biotic factors, fungal pathogens are major hindrance which restricts its marketable value. In the present study, four different varieties of litchi *viz*. Bedana, China, Deshi and Shahi were used. All the four varieties were assessed for the fruit color, the ratio of pulp and peel, pH of the fruit and Disease Incidence (DI) after 3rd, 6th and 9th day of storage. The color of the fruits of all varieties changes rapidly from 3rd to 6th day of storage, pulp content reduced more in the interval of 6th to 9th day, pH of the fruit ranged from 3.7 to 6.8 between the storage day and DI ranged to a value of 88.2 after 9th day of storage. The correlation states that the disease correlate positively to pH only up to 3rd day and after that increase in the value of pH of the fruit has no effect on the disease.

Keywords: Disease incidence, litchi, pH, pathogenic behavior, postharvest

Introduction
Litchi (*Litchi chinensis* Sonn.) is a Sapindaceae plant native to subtropical regions of southern China and northern Vietnam (Menzel *et al*., 1999) [10]. The crop has been farmed for at least 2000 years. The fruit was imported to Australia from Chinese miners very early in the mid-1800, but the import of good varieties with quarantine perspective did not occur until the 1920-30 (Greer, 1990) [5]. Some of the Chinese literature also mentioned about the perishability and ways to enhance the post-harvest life of the fruit. Litchi fruit established its minor industry with commercial planting estimated at around 350 growers and 1800 hectares after 1970 (Menzel *et al.*, 1999) [10].

Northern hemisphere has a high production of litchi, with areas including China, Taiwan Thailand, the USA, Israel, India and the area of South Africa including Australia, Mauritius, Madagascar, and other southern hemisphere countries are also covered by litchi belts. Australia exports litchi fruits to various Southeast Asian and European countries, accounting for 25% of world production (Menzel *et al*., 1999) [10]. In India, litchi is an important fruit crop having a remarkable domestic market and export prospective with 84,000 hectares of area and production of 594,000 metric tons (NHB, 2015) [8]. Due to its soil and temperature requirements, the fruit cannot be grown throughout the country. The type of soil and climate suitable for litchi production is found in Assam, Bihar, Jharkhand and West Bengal. The contribution of this fruit to the economics of farmers in the aforementioned states accounts for 78 percent of the country's total production. Bihar produces 40 percent of the entire litchi crop and roughly 38 percent of the entire area covered. Over 300,000 metric tons of litchi are grown on 32 thousand hectares of land in Bihar. Litchi varieties grown in India differ significantly depending on temperature and soil conditions. Bedana, China, Dehra Rose, Deshi, Early Bedana, Kasba, Kaselia, Late Bedana, Longia, Maclean, Manragi, Purbi, Shahi, and Swarna Rupa are among some of the popular grown varieties of Litchi.

Fruit output varies from year to year because to the varying flowering requirements (Paull and Duarte, 2011) [11]. Due to the presence of anthocyanin, the fruit is egg-shaped and has a grape-like pulp texture. It is succulent, aromatic, and juicy, with leathery and prickly red skin. The fruit is highly prized only in its fresh form and its marketable value decreases if it shows the sign of pathogen from outside. Aril, the fleshy edible component, encloses a small, black to brown seed that is susceptible to post-harvest diseases.

Although the pulp quality not depends on the color of the fruit up to some days of harvesting but the commercial quality of litchi only depends on fruit color, which is a major characteristic used to judge its marketable position. The change in color of the fruit pericarp is mainly due to loss in moisture and ultimately leads to deterioration after the harvest of the fruit (Huang and Scott 1985) [6]. Desiccation, micro cracking, and postharvest deterioration, in addition to browning in the pericarp, are some of the key constraints that limit the expansion of litchi commercial production. Micro-cracks, which are important cause of post-harvest loss developed during the development of fruit and postharvest handling can offer a harbor of entry for decay pathogens that inhabit the fruit surface (Sivakumar *et al*., 2005) [18]. The early varieties are more prone to the problem of fruit cracking in comparison to late cultivars. Fruit cracking is favored by low atmospheric humidity, high temperatures and hot winds during fruit development and maturity. Also the injury result into enhanced loss of moisture and respiration, which make it amenable to pathogen attack. Average level of postharvest losses varies between 5 to 30% in different crops (Ruiz Altisent, 1991) [16].

A wide range of fungi including *Alternaria*, *Aspergillus*, *Botryosphaeria*, *Fusarium*, *Penicillium*, *Peronophythora Pestalotiopsis*, *Phomopsis*, *Rhizopus* and *Trichoderma* can cause decay of litchi fruit during and after harvest through skin injury. *Botryodiplodia* infects fruit in the field or during harvesting or handling through the cut stem end. Scale insects and mealy bugs are among the insects that have infested the fruit and must be eradicated before it can be exported. Various fungicides, like benomyl, thiabendazole, iprodione and prochloraz has been used from time immortal to control the post-harvest diseases in litchi. But these fungicides due to potential oncogenic risks in many counties across worlwide have not been registered as a postharvest chemical. The present study is focused on different physiological changes that gives an indication about the attack of various postharvest plant pathogens on the fruit. Alternative decay control methods in litchi fruit are required, however, due to global consumer concern and upcoming regulatory changes in the usage of toxic chemicals.

Materials and Methods

Four commercially important varieties of litchi namely Bedana, China, Deshi and Shahi were used for the present study. A total of 40 fruits with similar shape and size and free of visible disease symptoms were used. The fruits were assessed for fruit color, pulp to peel ratio, pH of pulp and disease incidence. The pericarp redness was graded visually (Chu *et al*., 2004; Underhill and Critchley, 1993) [2, 19] as grade 1: < 10% red area; grade 2: 25% red area; grade 3: 50% red area; grade 4: 75% red area; and grade 5: > 90% red area. The fruits were peeled at the 3rd, 6th and the 9th days of storage. After separating, the peel and pulp weights were taken separately by using an electric balance and then the pulp to peel ratio was calculated. Pulp pH was determined by pH meter. Disease incidence was calculated as follows.

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits under study}} \times 100$$

A small bit of infected fruit pulp was washed with tap water and sterilized with 1% sodium hypochloride then again washed with tap water were placed in the center of petriplates filled with PDA (potato dextrose agar: 20 g of dextrose and 20 g of agar in 1000 ml of potato extract) and were placed at 28 ± 2 °C media for 3-4 days and were observed under microscope for the identification of pathogen associated with the litchi pulp.

Results and Discussion

Fruit color

All the fruits of four different varieties, attaining the color of full maturity were assessed for the change in their peel color in due time. The rating of color was 5 for all the varieties at 3rd day of storage. The color rating declines to 2-3 at 6th day of storage and it comes to 1 at 9th day of storage when the fruits were completely attacked by pathogens (fig 1a). The pericarp color of the fruit changes rapidly from 3rd to 6th day, from color rating of 5 to 2-3 and it reaches to nearly 1 at 9th day. The rate of change in color detonation was more between 3rd to 6th day as compared between 6th to 9th day. However, in the variety China and Shahi, the change in color was less as compared to the variety, Deshi and Bedana (fig. 1a). The color change of fruit pericarp is noticeable information for its quality determination, which plays a major role in its marketable value, although it does not affect more to the aril. Also, the stability of the anthocyanin molecules is sensitive to H^+ ion concentration in the fruit and their degradation along with other phenolic compounds are the resultant of enzymes such as PPO and anthocynase present in various pathogens. Change in the color of pericarp is also related to water loss or desiccation (Scott *et al*. 1982) [17]. Temperature, relative humidity and air currents are the major variables which controls the moisture loss from the fruit. The ability of the pericarp to resist moisture loss is affected by structure and compositions of the protective layers (Denna 1970) [3].

Pulp to peel ratio

The weight of pulp content and peel were taken separately by weighing balance and the ratio was estimated. The average ratio at 3rd day was 5.2, 5.6, 5.2 and 5.3 for the varieties Bedana, China, Deshi and Shahi, respectively (fig. 1b). It was maximum at 6th day with the respective mean value of 6.4, 6.6, 5.8 and 6.0. The ratio was found to be 4.3 for Bedana and Deshi varieties and 3.9 for China and Shahi varieties after 9th day of storage. The rate of increase in the ratio from 3rd to 6th day was less as compared to the rate of decrease in the ratio between 6th and 9th day of storage (fig. 1b). Deterioration of the fruit pulp leads to decrease in the activity of antioxidant and various defensive chemicals present in the pulp which are responsible for protection of the fruits from plant pathogens. Decrease in the pulp quantity makes the fruits amenable to attack by the pathogens, as pulp of various fruits have been designated as gel and the bitter, yellow liquid fraction have been proved to be antagonists against various plant pathogenic bacteria and fungus (Rodriguez *et al*., 2005) [15].

pH of the pulp

The pH of pulp was recorded between 3 to 4 after 3 days of storage. The value was recorded to be 4, 3.7, 3.9 and 4.03 for Bedana, China, Deshi and Shahi varieties respectively (fig. 1c). The pH increased by approx. one unit after 6 days and it was found to be between 6 and 7 after 9 days. It was found to be maximum of value 7.03 in the China variety and a lowest of 6.6 in the Deshi variety after 9 days (fig. 1c). The plant pathogens have different mechanisms to change the surrounding condition for their survival and alteration of host

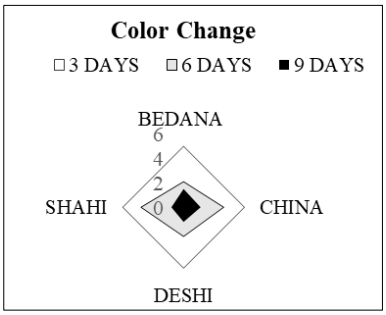
pH. In our study also, the pH of the pulp is increasing as the incidence of disease increases. The response to acid stress by *Saccharomyces cerevisiae* and *Aspergillus nidulans* have been seen (Maeda, 2012) [9]. Available nutrients, organic acids, ability of plant pathogens to remove ammonium ions from different salts decide the extent or magnitude of pH change (Prusky *et al*., 2001) [13]. Ambient pH condition in the host results in the activation of some of the particular genes that are responsible to cause disease in host (Kumar *et al*., 2018) [7]. In the present study, correlation between pH and Disease incidence (fig 1e) reveals that up to 3rd day of storage, the DI is significantly ($p \geq 0.05$) increase with a positive value of 0.74 and the increase in the pH value had a positive relation with the increase in DI. But after 6th day of storage the increase in pH value of the pulp is negatively correlated with DI for a value of -0.16 and the same case was observed after 9th day of storage, where the correlation value between the pH and DI was -0.79. In the present study the value of R^2 was found near to one when the relation was seen between color and the pH of fruits (fig. 1f). Several studies prove the mechanisms for change in pH of host tissue for the pathogen survival by expression of a particular subset of genes like production of citric acid, gluconic acid etc. (Prusky *et al*., 2004) [14]. Ambient pH for the pathogen is a regulatory prompt for progressions linked to pathogenicity (Kumar *et al*., 2018) [7]. Alkalization of the host environment during establishment by post-harvest pathogens is due to secretion of ammonia as a result of protease activity and deamination of amino acids (Prusky and Yakoby, 2003) [12] and the virulence of pathogens is accompanied by host alkalization (Alkan *et al*., 2008) [1]. It has been seen that the expression of endoglucanase gene AaK1 by necrotrophic fungi like *Alternaria alternate* is maximum at pH near to 6. This gene doesn't express at a lower pH and remain quiescent (Eshel *et al*., 2002) [4]. The gene pelB of *Colletotrichum gleosporioides*, is positively correlated with the increase in pH of the host tissue (Yakoby *et al*., 2000) [12] and only become functional when the value of pH become more than 5.7.

Disease Incidence

Four varieties of litchi i.e. Bedana, China, Deshi and Shahi were assessed for the incidence of disease. The pathogens attacking the fruits were grown on PDA medium and incubated for 3-4 days for assessment of their cultural morphology (fig 2) and the microscopic images of spores were taken and identified as *Alternaria* and *Rhizopus* (fig 3), based on their cultural and microscopic views. In Bedana variety the Disease Incidence (DI) was 20.3 and 59.37 after 3 and 6 days of storage. After 3 days, a minimum DI of 19.74 was found in china variety but it raises to 64.23 after 6 days of storage. DI of 21.13 and 21.88 was recorded in Deshi and Shahi varities respectively after 3 days. After 6 days of storage, DI for Deshi variety was 58.34 and for Shahi variety it was 65.45. DI was recorded highest after 9 days of storage (fig 1d). Bedana variety was found to be having highest DI of 88.25 after 9 days of storage followed by Deshi, Shahi and china with DI of 86.82, 85.10 and 84.12, respectively. This increasing trend of incidence in disease is only the result of interaction between pathogenic strain in an environment that favors their development. The increasing amount of disease incidence may be with the increase in number of present propagules of the plant pathogens.

Conclusion

The present study reveals about parallel changes in the normal physiology and increase in the number of plant pathogens in different varieties of litchi. The change in color of fruit occurs with some other process like increase in pH of the fruit which may be due to the resultant enzymatic activities. Positive value of correlation between H^+ ion concentration and DI due to presence of plant pathogens also states the similar information.



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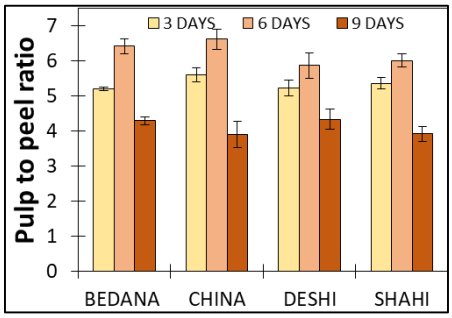

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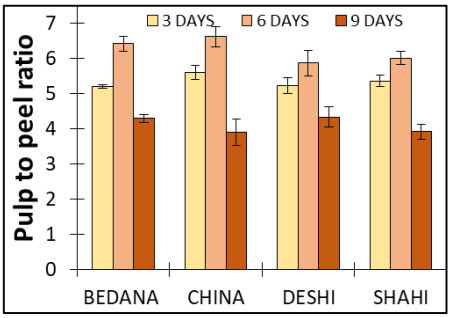

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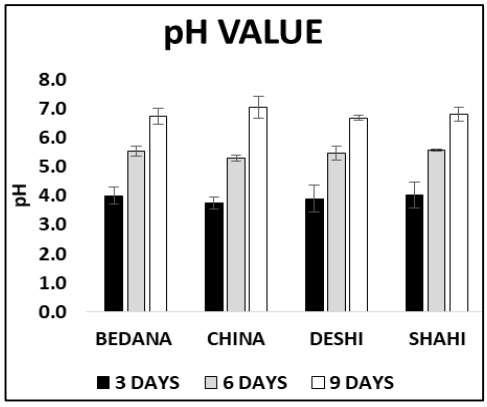

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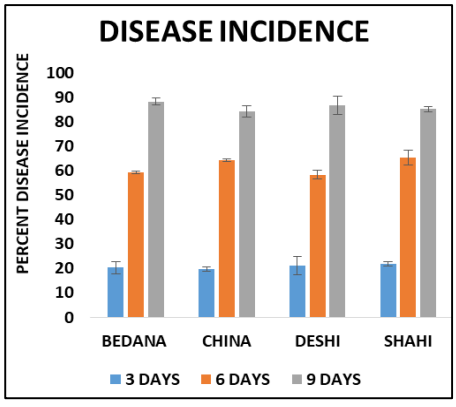

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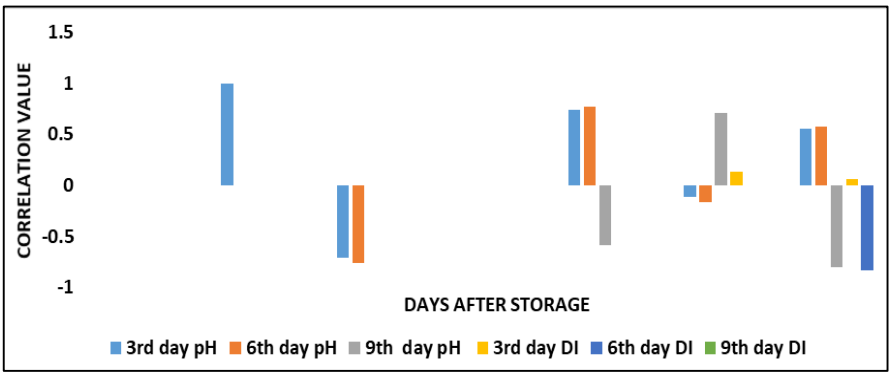

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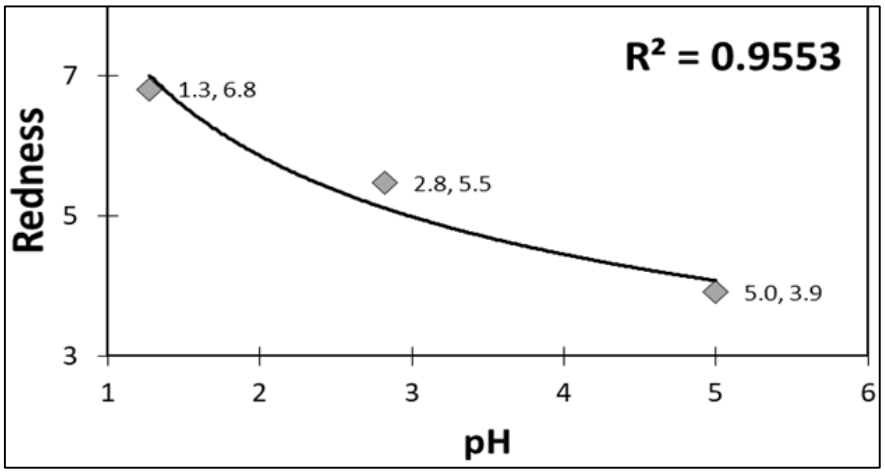

G

Fig 1: Graph showing a) color change, b) pulp to peel ratio, c) pH value in different days of storage, d) DI, e) correlation between pH and DI and f) relation between redness and pH

Fig 2: Pathogens grown on PDA media

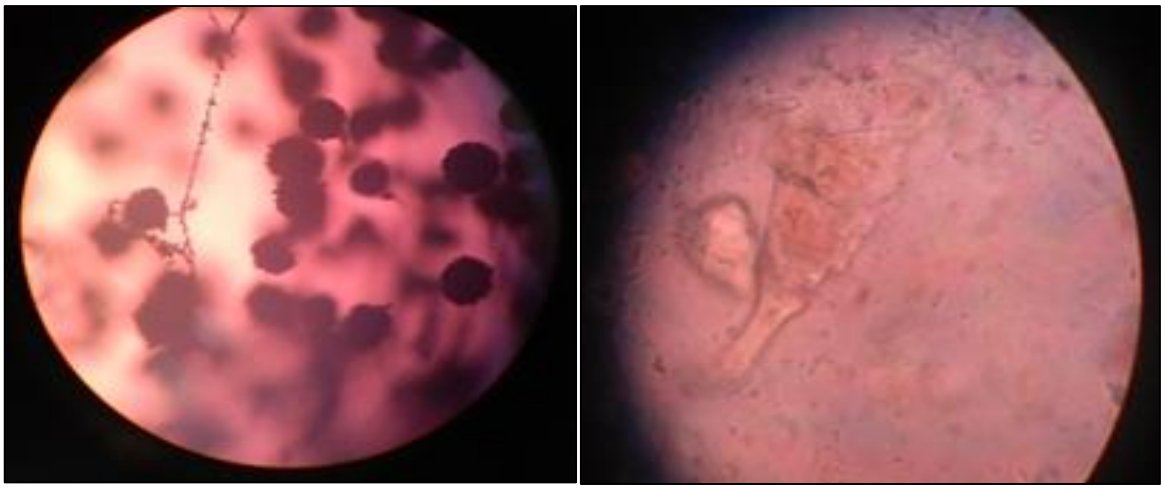

Fig 3: Microscopic photos of spores of different pathogens.

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