International Journal of Agricultural Sciences
Volume 17 | Issue 1 | January, 2021 | 48-53 ■ ISSN : 0973-130X
DOI:10.15740/HAS/IJAS/17.1/48-53
Visit us : www.researchjournal.co.in

RESEARCH PAPER

Effect of inoculum density of *Aspergillus niger* on quality of acid lime (*Citrus aurantifolia* swingle) during storage

Niren Majumdar* **and** Nakul Chandra Mandal[1]
Department of Plant Pathology, College of Agriculture, Tripura, Lembucherra (West Tripura) India
(Email: niren_majumder@yahoo.com)

Abstract : Acid lime (*Citrus aurantifolia* swingle) is available year the round either to use as fresh, stored for a short duration or in the preparation of pickles or other value added products to be consumed for a longer period. It is highly rich in vitamin C as antioxidant. It is infected by different fungal pathogens during transit and storage causing enormous loss both in quality and quantity. *Aspergillus niger,* an wound pathogen infect lime during storage. Inoculum density of a pathogen is critical for any successful infection and subsequent progression affecting fast deterioration in the quality of fruits. Eight different inoculum densities from 10^1 to 10^8 per ml of spore suspension were tested. Inoculum density determined the incubation period as revealed by 72 hours at 10^1 conc. and 48 hours at 10^2 conc. however, no discernible symptom appeared before 48 hours of incubation. Therefore, critical threshold limits for infection is below 10^1 spore conc. per ml. The loss in physical weight, vitamin C content, Titrable acidity and TSS of fruit juice reduced along with increased conc. of in inoculums load with maximum at 10^6 but with an obvious pH increase.

Key Words : Spore load, PDI, *Aspergillus niger*, Postharvest, Acid lime

View Point Article : Majumdar, Niren and Mandal, Nakul Chandra (2021). Effect of inoculum density of *Aspergillus niger* on quality of acid lime (*Citrus aurantifolia* swingle) during storage. *Internat. J. agric. Sci.*, **17** (1) : 48-53, **DOI:10.15740/HAS/IJAS/17.1/48-53**.
Copyright@2021: Hind Agri-Horticultural Society.

Article History : **Received :** 09.08.2020; **Revised :** 12.11.2020; **Accepted :** 15.12.2020

INTRODUCTION

Lime is a good source of vitamin C and has positive health values, especially bioactive substances and phenolic compounds. Among different citrus fruits, lime is much sour in taste as juice is too acidic, may be consumed as undiluted form (Berry, 2003). The distinctive natural volatile aroma of lime is also used in different other industries (Chisholm *et al.*, 2003). In case of *Tahiti* lime, vitamin C content of ranges from 39-62 mg per 100ml of juice (Ziena, 2000) while in Brazil in the early phase of storage it contains 31 mg per 100 ml of juice (Kluge *et al.*, 2003a). Postharvest decay is considered as the limiting factor for long period storage (Schirra *et al*., 2000) particularly fungal diseases (Davis and Albrigo, 1994). Fresh and healthy (Nurulhuda *et al*., 2009) fruits have special appeal to consumers but diseased units are unmarketable. Due to their higher water content and nutrient composition, citrus fruits become much susceptible to infection by microbial pathogens during the period between harvest and consumption (Tripathi and Dubey, 2003). Under high humid conditions,

*** Author for correspondence :**
[1]Department of Plant Protection, Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, Birbhum (West Bengal) India

postharvest diseases accounts for about 50 per cent of losses in stored fruits (Agrios, 2005 and FAO, 2002).

Among different species of *Aspergillus, A. niger* is the most dominant species under important tropical postharvest storage pathogen of lime, although rarely found in the field. *Aspergillus* species dominate in the tropics, whereas *Penicillium* species are more common in temperate zones (Hocking, 2006). Both relatively high temperature of 25–40°C and high relative humidity favour the growth of these organisms but optimum temperature for rapid decay and spread noticed at 30–35°C (Milind, 2008). *Aspergillus niger* is considered as polyphagus pathogen with wide host diversities like apples, pears, peaches, citrus, figs, strawberries, mangoes and melons grapes etc. (Dasgupta and Mandal, 1989 and Snowdon, 1991). *A. niger* is a wound pathogen but can also infect at the weak pedicel point (Dasgupta and Mandal, 1989) under higher density of spore load (Valero and Serrano, 2010). Inoculum potential of *A. alternata* on harvested apple fruit was determined and found to be 10^6 conidia per ml (Verma, 2004) but a lower density, from a 5-7 days old culture on PDA, 10^5 per ml conidial suspension can cause successful infection on citrus leaves (Kakvan *et al.,* 2012). Eden *et al.* (1996) highlighted the practical importance of reducing the inoculum level to minimize infection and for postharvest caused by wound pathogens. Inoculums as spore density seems has not been investigated under tropical condition, therefore, the present investigation was conducted.

MATERIAL AND METHODS

The fruit:

Fresh and apparently healthy lime (*Citrus aurantifolia*) fruits were collected from local Sriniketan market, Bolpur, West Bengal with uniform, size, shape and maturity. Fruits were surface sterilized with 0.1 per cent Mercuric chloride for 1 minute followed by three times washing with sterile distilled water in order to remove the mercuric chloride residue.

The pathogen and isolation:

The pathogen *Aspergillus niger* was previously isolated on standard PDA medium from the naturally infected fruit. Identified pathogen was purified as pure cultures through single spore culture technique. The pathogen was maintained on PDA as pure culture in slants at 4ºC for future studies. Ten days old culture was used for this experiment.

Preparation of spore suspension:

The spores of *A. niger*, was used as inoculm, were collected from 10 days old cultures grown on the PDA media as described above for the experiment. Mycelial mats containing spores was taken within a culture tube containing sterile distilled water and thoroughly shaken along with tween 20 for even distribution in the water and followed by a passage through a thin glass wool filter to remove hyphal growth. Initially a suspension of higher spore density was prepared and further diluted by serial dilution technique for obtaining lower spore concentrations (10^1 to 10^7 spores/ml). Each conc. was standardized by checking with a haemacytometer. Fresh spore suspensions were used for inoculation.

Inoculation and incubation of lime fruits:

Surface sterilized apparently healthy lime fruits were artificially injured with the help of sterile scalpel in the equatorial region of the fruit with a size of about 3mm. Spore suspension of different concentrations were taken in separate sterile syringes with wide pore needle and individual fruits were inoculated by placing one drop of spore suspension on the injured site. Immediately after inoculation fruits were placed within a sterile polypropylene bag alongwith sterile water soaked cotton swab to maintain the high humidity. All activities were conducted in front of a laminar flow chamber to avoid contamination. Inoculated fruits were incubated at 27 ±1°C for upto 10 days, although after 24 hours of incubation polypropylene bags were perforated.

Different treatments were as follows:

T_1 = Control without injury, T_2= Control with injury, T_3= Spore concentration of 10^1, T_4= Spore concentration of 10^2, T_5=Spore concentration of 10^3, T_6= Spore concentration of 10^4, T_7=Spore concentration of 10^5, T_8= Spore concentration of 10^6, T_9= Spore concentration of 10^7, T_{10}=Spore concentration of 10^8.

PDI (% disease index) calculation:

0-9 scale of disease have been adopted for calculating the PDI . The scale is as follows:

Disease grade	Per cent of fruit surface infection
0 :	No symptom/infection
1 :	>5 % infection
2 :	5-10% of infection
3 :	10-15% of infection

4 : 15-25% of infection
5 : 25-40% of infection
6 : 40-60% of infection
7 : 60-80% of infection
8 : 80-99% of infection
9 : 100% of infection.

$$PDI = \frac{\sum \text{All disease ratings}}{\text{No. of observation x Maximum disease grade}} \times 100$$

Determination of quality of fruit:

Physiological weight loss per cent:

Physiological weight loss per cent of infected fruit was assessed at 10th days and losses in weight were calculated by the following formula:

$$\text{Physiological wt. loss \%} = \frac{W_1 - W_2}{W_1} \times 100$$

where, W_1 = Weight of the fruit recorded at the time of inoculation

W_2 = Weight of fruit recorded at 10th days of inoculation.

Estimation of vitamin C content:

Vitamin C content in fruit juices was estimated by volumetric method (Thimmaiah, 1999). Five ml of standard ascorbic acid (100 µg/ml.) was taken in a conical flask containing 10 ml of 4 per cent oxalic acid and was titrated against the 2,6- dichlorophenol indophenols dye. The appearance and persistence of pink colour was taken as end point. The amount of dye consumed (V_1 ml) is equivalent to the amount of ascorbic acid. Five ml of sample (prepared by taking 5ml of juice in 100 ml 4% oxalic acid) was taken in a conical flask having 10 ml of 4 per cent oxalic acid and titrated against the dye (V_2 ml). The amount of ascorbic acid was calculated using the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{0.5 \text{ mg}}{V_1 \text{ml}} \times \frac{V_2 \text{ml}}{15 \text{ ml}} \times \frac{100 \text{ ml}}{\text{wt. of sample}} \times 100$$

TSS:

ATAGO digital pocket refractometer was standardized and TSS of samples was recorded using this refractometer.

Titrable acidity:

Titrable acidity of juice was estimated by titration with 0.1 N NaOH following AOAC (1990) method using the formula:

$$\text{Titrable acidity} = \frac{\text{Eq. wt. of acid x Normality of NaOH (0.1) x 100}}{\text{Weight or vol. of sample x 1000}}$$

pH estimation:

Samples of juice were collected by squeezing the fruit and passing through muslin cloth in order to remove the seeds and pulp particles. The pH of the sample was determined by the digital pH meter with its electrode after standardization. Average value of pH of three replications was considered for the treatment.

Statistical analysis:

All experiments were conducted in a Completely Randomized Design with three repetitions, for each treatment. The statistical analysis of the results was conducted by analysis of variance (ANOVA) in MS excel sheet.

RESULTS AND DISCUSSION

The Table 1 indicated that infection progressed only

Table 1 : Progress of infection* of *A.niger* under different spore load on lime fruits

Treatments	PDI								
	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs	192hrs	216hrs	240hrs
T_1	0	0	0	0	0	0	0	0	0
T_2	0	0	0	0	0	0	0	0	0
T_3	0	7.41	7.41	11.11	29.63	51.85	59.26	62.96	74.07
T_4	3.7	7.41	7.41	14.81	37.04	59.26	70.37	83.33	92.59
T_5	3.7	11.11	11.11	14.81	29.63	55.56	62.96	81.48	85.19
T_6	3.7	11.11	12.11	22.22	55.56	72.22	92.59	100	100
T_7	7.41	11.11	22.22	44.44	59.26	70.19	82.19	88.19	92.59
T_8	11.11	11.11	18.52	37.04	55.56	66.67	74.67	85.19	96.3
T_9	11.11	11.11	22.22	59.26	66.67	81.48	88.89	96.3	96.3
T_{10}	11.11	14.81	25.93	55.56	66.67	85.19	96.3	100	100

*PDI values # No visible infection detected after 24 hours of incubation

in the presence of inoculum, suggests the fruits were free from the *A. niger*. There were steady progresses of infection of all the treatments as reflected in the PDI values, although sometimes in the early phase progress of the disease could not be noticed. Probably all the spores were not germinated at a time or pathogen took some time to overcome the host resistance barrier. Gandon and Michalakis (2000) showed that once quantitative plant resistance is eroded, pathogens exhibited greater virulence not only on the resistant host, but also on fully susceptible hosts. Fruits were totally infected within a period of 9- 10 days of incubation. Self inhibition of infection was not noticed even in T_{10} having highest concentration of inoculum .When the PDI values of all the treatments were compared after 168hs of incubation maximum rotting was found in both T_7 and T_{10} with PDI value 85.19. There was practically no difference between T_9 and T_{10}. Treatment T_3, T_4 and T_5 can be considered as same as there were almost no difference among treatments. This experiment of infectivity titration may help to determine the shelf-life of the naturally infected fruit. More over for any other experiment inoculums dose of T_4 –T_6 may be considered optimum.

The behaviour of infection *A. niger* is similar to that of *Rhizopus stolonifer* on tomato as both of them can infect mature and mature-green fruits of acid lime and tomatoes, respectively, even at the lowest concentration of 10^1 spores per ml (Silvia Bautista *et al.,* 2008). Similarly, spore conc. at 10^4 spores ml^{-1} and 10^6 spores ml^{-1} of *C. gloeosporioides* have similar effects on infection but *M. fructicola* behaved differently. Lesion size too was relatively larger at 10^6 in *M. fructicola* as compared to 10^4 concentration (Plate 1).

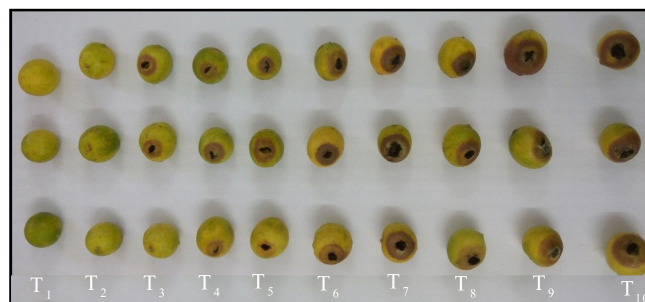

Plate 1 : Spore load of *A. niger* on lime at 5th day of incubation

Post-infectional changes pertaining to physical, biochemical and nutritional aspects caused due to major post-harvest microbial pathogens have been extensively compiled (Dasgupta and Mandal, 1989). The weight losses in fruits over a period of time may be due to loss

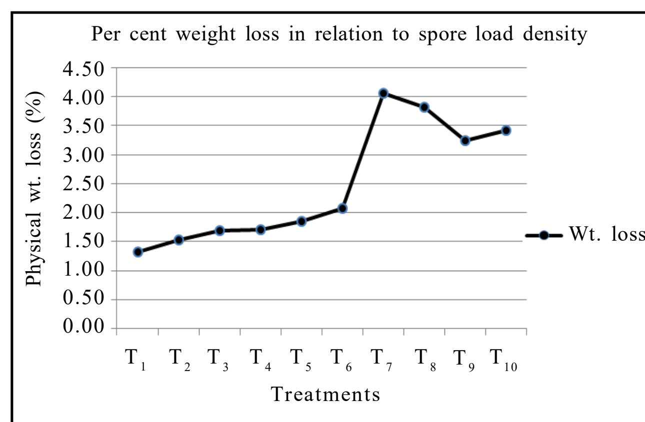

Fig. 1 : Per cent physical weight loss in different spore load of *A. niger* (10DAI)

Table 2 : Effect of fruit quality due to spore load of *A.niger* on lime at 7 day of incubation

Treatments	pH	Titrable acidity (%)	Vitamin- C (mg/100ml)	TSS(^{0}Brix)
T_1	2.29	6.87	25.58	7.32
T_2	2.32	6.85	23.56	7.25
T_3	2.33	6.76	21.18	6.86
T_4	2.34	6.68	21.65	6.88
T_5	2.35	6.34	19.48	7.01
T_6	2.38	6.11	15.61	5.94
T_7	2.41	6.23	14.03	6.03
T_8	2.56	5.83	13.30	5.89
T_9	2.49	6.14	13.53	5.78
T_{10}	2.51	6.05	12.50	5.51
S.E.±	0.03	0.20	0.87	0.08
C.D. (P=0.05)	0.10	0.59	2.56	0.24

in moisture content by evaporation and in infected fruits by hydrolysis of tissues followed by either leaking or both leaking of juice and evaporation. It was found that treatments T_1 to T_5 almost have no impact on moisture loss but it sprang up suddenly at T_6 treatment and gradually declined to some extent in other concentrations (Fig.1). The correlation studies between PDI and moisture loss showed positive relationship with r value = 0.60 .

Table 2 indicated that pH gradually increased with the increase of inoculums density and thus, favouring the quick growth and multiplication of the pathogen. This high acidic condition of lime juice is known to be due to 5-6 p.c citric acid conc. There was no statistical difference among the treat of T_1 to T_6. Similarly, excepting treatment T_8, other treatment have statistically similar values. Our findings corroborate with the findings referred by Mandal and Dasgupta, 1989 in case of infected orange with *Alternaria citri, Geotrichum candidum* and *Penicillium digitatum.*

Titrable acidity p.c. have been found to be declined along with higher spore density but such decline is not statistically significant excepting T_8. Treatment T_8 showed the critical point where pH increase and acidity decrease significantly. Damaram 2013 also observed that acidity content of inoculated tomato fruits with *F. pallidoroseum* progressively decreased in relation to incubation period. Mandal and Dasgupta, 1989 compiled the data on reduction in TSS in lemon, sweet orange, infected with different fungi. In lime – *A. niger* also similar results were reported (Reddy and Laxminarayana, 1984).

The concentration of vitamin C gradually decreases over time but become faster with infection. The vitamin C content decreased along with increase of inoculum density. Ascorbic is the precursor of vitamin C. Similar trend of the results was reported in different host – pathogen systems like Papaya- *A. flavus* and *F. moniliforme* (Singh *et al*., 2010), mango and papaya- *A. niger* (Ghosh *et al*., 1966), tomato - *A. niger*, *A. flavus*, *Alternaria alternata*, *A. solani* and *Fusarium oxysporium* (Ogaraku *et al*., 2010). Kinnow- *Alternaria alternata, Botryodiplodia theobromae, G. candidum, Penicillium digitatum* and *P. italicum* (Sharma *et al*., 2011). The decline of ascorbic acid content in many other host - pathogen systems has also been compiled by Dasgupta and Mandal (1989).

There was a declining trend of TSS content in relation to increasing concentration of spore load. The correlation co-efficient value r = -0.75. The results revealed significant decrease in fruit acidity and ascorbic acid content as compare to uninoculated healthy fruits. Tandon, 1970 found that ascorbic acid of mango pulp was decreased due to *A. niger*. Similar result observed by citrus (Agrawal and Ghosh, 1979), musambi (Singh and Sinha, 1982).

Conclusion:

The critical threshold limits for infection of *A.niger* to acid lime is below 10^1 spore conc. per ml. The loss in physical weight, vitamin C content, Titrable acidity and TSS of fruit juice reduced along with increased conc. of in inoculums load with maximum at 10^6 but with an obvious pH increase.

REFERENCES

Agrawal, G.P. and Ghosh, K. (1979). Post inection changes in as ascorbic content in lemon, musambi and orange fruits infected by colletotrihum gloeosporioides. *Indian Phytopath.*, **32** : 108-109.

Agrios, G. N. (2005). *Plant pathology*, 5th Ed. Elsevier Academic Press USA, 383- 557pp.

AOAC (1990). *Official methods of analysis of the association of official analytical chemists* 15th Ed. Virginia, Washington D.C., U.S.A.

Berry, R.E. (2003). *Citrus fruits: Limes, Encyclopedia of food sciences and nutrition*. In: Caballero B. (Ed.). Academic Press, Oxford. 1368-1372pp.

Chisholm, M.G., Jell, J.A. and Cass, D.M.J. (2003). Characterization of the major odorants found in the peel oil of *Citrus* reticulate Blanco cv. Clementine using gas chromatography olfactometry. *Flavour Frag. J.,* **18** : 275–281.

Damaram (2013). Investigation on fusarium fruit rot [*Fusarium pallidoroseum* (cooke) sacc.] of tomato (*Lycopersicon esculentum* mill.) and its management. Anand Agricultural University, Anand, Gujarat (India).

Dasgupta, M.K. and Mandal, N.C. (1989). *Postharvest pathology of perishables*. Oxford and IBH Publishing Company Private Ltd.pp300-308.

Davies, F.S. and Albrigo, L.G. (1994). *Environmental constraints on growth,development and physiology of citrus*.Citrus: Crop Production Science in Horticulture, **2**.CAB international Wallingford,UK.52-82pp.

Eden, M.A., Hill, R.A., Beresford, R. and Stewart, A. (1996) . The influence of inoculum concentration, relative humidity,

and temperature on infection of greenhouse tomatoes by Botrytis cinerea. *Plant Pathology,* **45**: 798-806.

Gandon, S.Y. and Michalakis (2000). Evolution of parasite virulence against qualitative or quantitative host resistance. *Proceeding of the Royal Society of London,* **267** (B) : 985–990.

Ghosh, A.K., Bhargava, S.N. and Tandon, R.N. (1966). Studies on fungal diseases of some tropical fruits. IV. Post-infection change in ascorbic acid contents of mango and papaya. *Indian Phytopath.,* **19** : 262-268.

Hocking, A.D. (2006). *17- Aspergillus and related teleomorphs. Food dpoilage microorganisms.* A volume in Wood head Publishing Series in Food Science, Technology and Nutrition, 451-487

Kakvan, N., Zamanizadeh, H., Morid, B., Taheri, H. and Hajmansor, S. (2012). Study on pathogenic and genetic diversity of *Alternaria alternata* isolated from citrus hybrids of Iran, based on RAPD-PCR technique. *European J. Experimental Biol.*, **2** (3): 570-576.

Milind, S. Ladaniya (2008). P*ostharvest diseases and their management. Citrus fruit biology, Technology and Evaluation*. Academic Press.2008, Pages 417-449, XVIII-XIX.

Nurulhuda, M.S., Latiffah, Z., Baharuddin, S. and Maziah, Z. (2009). Diversity of *Fusarium* species from vegetables fruits. *J. Malaysian Appl. Biol.,* **38** (1): 43-47.

Ogaraku, A.O., Alanana, J.A. and Omananyi, P.O. (2010). Decay of tomato (*Lycopersicum esculentum* Mill) and vitamin C content of infected fruits in keffi, nasarawa State. *PAT,* **6** : 91-98.

Reddy, S.M. and Laxminarayana, P. (1984). Post infection changes in ascorbic acid contents of mango and amla caused by two fruit-rot fungi. *Curr. Sci*., **53**: 927-928.

Schirra, M., D'hallewin, G., Ben-Yehoshua, S. and Fallik, E. (2000). Host-pathogen interactions modulated by heat treatment. *Postharvest Biology & Technology,* **21**:71-85.

Sharma, R.N., Maharshi, R.P. and Gaur, R.B. (2011). Post-infectional changes in kinnow (*Citrus deliciosa*) fruits Incited by post-harvest fungal rot pathogens. *J. Mycol. Pl Pathol.,* **41** : 483-486.

Silvia Bautista-Banos, Miguel, G.,Velaquez-Del Valle, Ana, N. Hernandez-Lauzardoa and Essaid, Ait Barka (2008). The *Rhizopus stolonier*-tomato interaction. *Plant-Microbe Interactions,* 269-289 ISBN: 978-81-308-0212-1 Editors: E. Ait Barka and C. Clément.

Singh, A. and Sinha, K.K. (1982). Biochemical changes in musambi fruit inoculated with species of aflotoxin production *Aspergilli. Curr. Sci.,* **51**: 841-842.

Singh, P., Mishra, A.K. and Tripathi, N.N. (2010). Pathogenic and cultural properties of post-harvest pathogens of papaya. *Ann. Plant Protect. Sci.,* **18** : 427-433.

Snowdon, A.L. (1990). A colour atlas of post-harvest diseases and disorders of fruits and vegetables. Vol. **1** Wolfe scientific Ltd. London 672 pp.

Tandon, R. N. (1970). Certain problems of post harvest diseases of fruits and vegetables. *Indian Phytopath.,* **13**: 1-15.

Thimmaiah, S. K. (1999). *Standard methods of biochemical analysis*, Kalyani Publishers, Noida (U.P.) India.

Tripathi, P. and Dubey, N. (2003). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biol. Technol.,* **32**: 235–245.

Valero, D. and Serrano, M. (2010). *Postharvest biology and technology for preserving fruit quality.* CRC press Taylor and Francis group.pp. 62.

Verma, U.K. (2004). Management of postharvest diseases of tomato (*Lycopersicon esculentum* Mill.) with special emphasis on biological control. Ph.D Thesis, Botany Department, Lucknow University, Lucknow (U.P.) India.

Ziena, H.M.S. (2000). Quality attributes of Bearss seedless lime (*Citrus latifolia* Tan) juice during storage. *Food Chem.,* **71** : 167-172.

WEBLIOGRAPHY

FAO (2002). *www.fao.org/decrep/t0073e/too73e00.html contents.*