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INFLUENCE OF pH ON THE GROWTH OF *ALTERNARIA CITRI* ON CITRUS FRUIT JUICE

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Abstract. **The influence of pH of juice on the extent of growth of *Alternaria citri* Ellis & Pierce that causes 'Black-rot' in stored citrus fruits was studied. The study included fruit juice of 5 commercial citrus cultivars: 'Feutrell's Early' and 'Kinnow' mandarins (*Citrus reticulata* Blanco); 'Valencia' oranges (*Citrus sinensis* Osbeck); 'Marsh' grapefruit (*Citrus paradisi* Macf) and 'Eureka' lemon (*Citrus limon* Burm). *Alternaria citri* showed significantly more and rapid growth on the juice from 'Kinnow' and 'Feutrell's Early' having pH about 4.0 as compared to the juice of 'Valencia' oranges, 'Marsh' grapefruit and 'Eureka' lemon having pH 3.7 or below. In confirmatory experiments, the pH of juice of all cultivars under investigation was adjusted to 4.5 and then growth of *A. citri* was measured for 12 days at a temperature of 29 ± 1°C. It was found that in fruit juice of all the cultivars, the growth increased substantially and thus showed pH dependence.**

Black-rot is the cause of serious losses of citrus fruits when stored even after due protection treatments against water loss and penicillium-rots have been given. The water loss is controlled by wax treatment (1), or using certain lining materials (2) while *penicillium-rots,* i.e. blue mold, *Penicillium italicum* Wehmen and green mold, *Penicillium digitatum* Sacc. are protected against with the treatment of fungicides like thiabendazole (2), imazalil (7), and sodium-0-phenyl phenate (8) etc. Occurrence of black-rot during storage, though insignificant at 5°C after 6 weeks, is highly significant at 7.5-10°C. Beyond 6 weeks storage, black-rot is highly significant at all storage temperatures (9).

There is a great need io investigate different methods and fungicides that could control the problem of black-rot in stored citrus fruits. It is reported that *A. citri* rot can significantly be controlled in 'Ellendale' tangors and 'Washington Navel' oranges by the application of imazalil, tridemorph, or gauzatine (9). An antifungal complex derived from *Bacillus subtilis* AECL-69 significantly controlled black-rot in 'Valencia' oranges and 'Kinnow' mandarins (3,4). It has also been reported that 2,4-dichlorophenoxyacetic acid (2,4-D) + gauzatine effectively controls the rot in 'Valencia' oranges (6). We earlier reported that antibiotic F also effectively checked the sporulation in the artificially inoculated fruit (5). During our studies, we found that some batches of fruit gave results different from other batches, therefore, a study was made to grow *A. citri* in juice agar media. It was found that irrespective of citrus varieties, there was a relationship between the pH of the juice and growth rate of *A. citri* on juice agar media. When pH of the juice was artificially maintained it was apparent that except on 'Eureka' lemon, the fungal growth on the juices was comparable.

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Materials and Methods

A random sample of five fruits for each cultivar was taken. The fruit was washed thoroughy with water, dried in air, dipped in rectified spirit and then flamed. The fruit was peeled and juice extracted using a press machine under aseptic conditions. About 100 ml juice was saved for growth purposes, and sterilized at 15 lb for 15 min. A glass electrode was used to record pH of juice from each sample. Water agar gel, 4 percent, was prepared and sterilized by autoclaving at 15 lb for 15 min. The sterilized agar was kept at 40°C. Water agar gel and the fruit juice were mixed in equal volumes. About 25 ml of juice-agar was poured per petri plate of 100 mm diameter and the medium was allowed to solidify. *Alternaria citri* V4(4) was grown for 3-5 days on 2 per cent malt extract agar where the fungus formed a uniform layer of sporulating mycelial mat. Inoculum plugs 10 mm in diameter were cut from the fungal mycelial mat. One plug was placed in the middle of each plate of juice agar. Each juice had six plates as replicates. The plates were incubated at 29°C. The radial growth of each plug on medium prepared from juice of different cultivars was measured up to 12 days for comparison. In another experiment, the juice from different cultivars were collected and the pH of each juice extract was set at 4.5 using concentrated KOH. *A. citri* plugs were grown on these juice-agar plates as described above and the radial growth was measured for 12 days for comparison.

Results and Discussion

Growth of fungus *A. citri* V4 on juice-agar media prepared by using juice extracts of different cultivars is shown in Table 1. Lemon juice that had pH 2.6 supported far less growth of fungal mycelial plug which attained a diameter of 20 mm after 12 days. The pH of the juice extract could vary depending upon the state of fruit ripening. Some fruit, especially the older, tend to have higher pH than the freshly harvested fruit. Fruit juice extract from freshly harvested 'Kinnow' that had pH around 3.7 supported less growth, i.e. 66 mm diameter as compared to juice having pH around 4.1 and that had 82 mm diameter of growth of the fungus plug after 12 days.

A linear relationship was observed when the initial pH of the juice-agar medium or pH of juice at harvest was plotted versus the diameter of mycelial plug after 12 days growth period (Fig. 1), irrespective of juice origin. It was assumed that growth differences were due to pH. The presumption that the differences in growth were because of juice pH got support from further experimentation. The pH of juice from different cultivars was adjusted to 4.5 with KOH. The extent of fungus growth is shown in Table 2. The juices from 'Valencia', 'Kinnow', 'Marsh' and 'Feutrell's Early' supported equal extent of growth, except

Table 1. Growth of *Alternaria citri* on citrus fruit juice agar media.

Type of fruit	Juice pH (at harvest)	Growth of *Alternaria citri* V4 plug on day				
		0	3	6	9	12
		mm				
Orange (*Citrus sinensis* Osbeck cv: Valencia)	3.7	10	18	33	50	66
Mandarin (*Citrus reticulata* Blanco cv: Kinnow)	3.7	10	20	35	50	66
Mandarin (*Citrus reticulata* Blanco cv: Feutrell's Early)	4.1	10	30	50	60	82
Grapefruit (*Citrus paradisi* Macf cv: Marsh)	4.5	10	30	50	60	85
Lemon (*Citrus limon* Burm cv: Eureka)	3.6	10	20	35	55	65
	2.6	10	10	14	18	20

Table 2. Growth of *Alternaria citri* on citrus fruit juice agar media adjusted to pH 4.5.

Type of fruit	Juice pH (adjusted)	Growth of *Alternaria citri* plug on day				
		0	3	6	9	12
		mm				
Orange (*Citrus sinensis* Osbeck cv: Valencia)	4.5	10	30	60	80	87
Mandarin (*Citrus reticulata* Blanco cv: Kinnow)	4.5	10	30	60	75	85
cv: Feutrell's Early	4.5	10	30	60	80	85
Grapefruit (*Citrus paradisi* Macf cv: Marsh)	4.5	10	26	50	75	85
Lemon (*Citrus limon* Burm cv: Eureka)	4.5	10	21	32	45	60

'Eureka' lemon juice that had extreme acid contents with the initial pH 2.6. This indicates that the incidence of black-rot in citrus fruit is likely to be dependent upon the pH (sweetness) of the fruit at the time of storage. It can be recommended that early harvesting of fruit at the optimum stage of maturity will significantly reduce the problem of black-rot during storage.

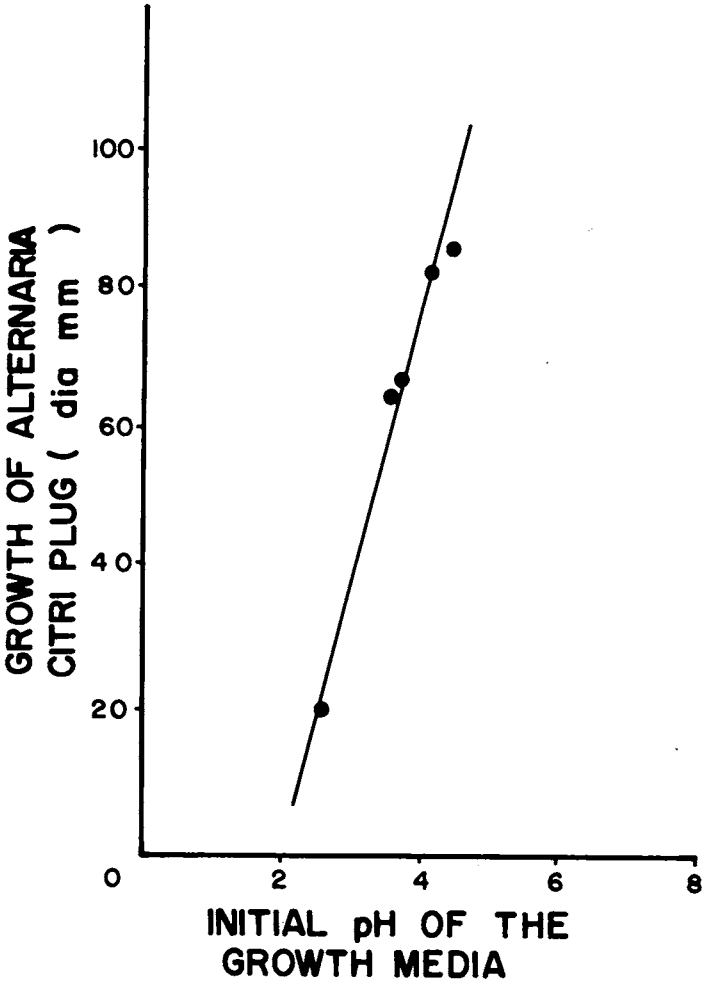

Fig.1 Relationship between ph of the medium and growth of alternaria citri.

Literature Cited

1. Ahmad, M., Z. M. Khalid and W. A. Farooqi. 1979. Effect of waxing and lining material on the storage life of citrus fruits. Proc. Fla. State Hort. Soc. 92:237-40.
2. Farooqi, W. A., M. Ahmad, A. M. Hussain and M. J. Qureshi. 1975. Effect of thiabendazole and packaging materials on Kinnow mandarin during storage. The Nucleus 12:23-25.
3. Farooqi, W. A., G. A. Shaukat, M. A. Malik, and M. S. Ahmad. 1979. A study on the control of internal blackening in oranges. Punjab Fruit J. 37:51-55.
4. Farooqi, W. A., G. A. Shaukat, M. A. Malik, and M. S. Ahmad. 1981. Studies on the inhibition of *Alternaria citri* in stored citrus fruits. Proc. Fla. State Hort. Soc. 94:279-280.
5. Farooqi, W. A., G. A. Shaukat, M. A. Malik, and M. S. Ahmad. 1984. Control of black-rot in inoculated citrus fruit. Citrus Ind. 66:62-66.
6. Gillespie, K. J. 1981. Chemical control of *Fusarium* and *Alternaria* rots in long term storage of citrus. S. Austral. Dept. of Agr. (Pl. Ind. Div.) Rpt. 8/81.
7. Kaplan, H. J. and B. A. Dave. 1979. The current status of imazalil: A postharvest fungicide for citrus. Proc. Fla. State Hort. Soc. 92:37-43.
8. Long, J. K. and E. A. Roberts. 1958. The phytotoxic and fungicidal effects of sodium-o-phenylphenate in controlling green mould wastage in oranges. Austral. J. Agr. Res. 9:609-628.
9. Rippon, L. E. and Wild, B. L. 1975. Ellendale tangors for export. Agr. Gaz. N.S.W. Austral. 86 (4) Aug.