Experimental Control of Citrus Fruit Decay with 1-Piperidino-2-Phenyl-3-Butanone Hydrochloride

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The fungi most commonly found attacking citrus fruits are Penicillium italicum (blue mold), Penicillium digitatum (green mold), and Phomopsis citri (stem-end rot). After long storage still other fungi, for example, Alternaria citri and Diplodia natalensis, may be involved (Wolf and Wolf, 1947). The importance of proper regulation of temperature and humidity for the control of mold growth during transportation and storage is well recognized (Lauriol, 1951). However, it is not always possible to prevent fungus activity by storage at low temperature alone. Hence, the treatment of fruit with chemical agents is practiced as a companion measure (Winston, 1935; Ramsey et al., 1944; Childs and Siegler, 1944; Lauriol, 1952; Reddish, 1954).

During investigation of the antimicrobial properties of a series of 8-piperidinopropiophenones (Florestano et al., 1957), one of the compounds, 1-piperidino-2-phenyl-3-butane hydrochloride, was found to possess marked activity in vitro against a number of fungi pathogenic to animals, as well as against Chaetomium globosum, involved in mildew-rotting of fabrics, and against Sclerotinia fructicola, the cause of brown-rot in stone fruits. In addition, the compound showed significant activity against Micrococcus pyogenes var. aureus and Escherichia coli.

1-Piperidino-2-phenyl-3-butane hydrochloride, with a molecular weight of 267.8 and melting point of 166 to 168°C, is a heat labile, white crystalline substance, highly soluble in water and ethanol. Although originally investigated for its efficacy in the treatment of dermatophytoses, the high degree of activity shown by the compound against fungi pathogenic to plants suggested its possible use as an agricultural fungicide. The present report deals with the effectiveness of this derivative in the control of fungus growth on citrus fruit.

Experimental Methods

For this study, oranges bought on the open market were used. Preliminary to testing, each orange was hand-washed in a mild solution of trisodium phosphate followed by thorough rinsing in tap water. The fruit was left exposed to dry overnight at room temperature.

Experiment 1. To test the efficacy of 1-piperidino-2-phenyl-3-butane hydrochloride as a growth inhibitor of naturally (air-borne) occurring fungi, lots of 30 oranges were sprayed, respectively, with alcoholic solutions containing 0.5, 2, and 5 per cent of the compound. For purpose of comparison, an additional group of 30 oranges was treated with an 8 per cent aqueous solution of borax (sodium borate). A separate lot of 30 untreated oranges was included as a control. To insure adequate contact with the chemicals, each lot of oranges to be treated was arranged in a single layer in a wire basket (3½ in. mesh) and subjected from all angles to a strong, continuous spray of 1 min duration. After treatment, respective lots were transferred, stem-end up, to sterilized porcelain trays and stored at room temperature for a period of 4 weeks. The oranges were examined at weekly intervals, noting (1) the number of sound fruit, (2) the number of oranges with visible fungus growth, (3) the number of decayed oranges with no obvious fungus growth, and (4) the number showing any surface effect that could be attributed directly to the chemical per se.

Results are presented in table 1. Since no direct damage to the rind through chemical action was appar-

\[ \text{Streptococcus liquefaciens} \text{ inoculated into orange concentrate.} \text{ Appl. Microbiol., 3, 104–106.} \]


ent, only the percentages of sound oranges and oranges with visible fungus growth are recorded, the difference from the total number in each lot consisting, therefore, of oranges undergoing decay with no perceptible fungus growth. From the results obtained, 1-piperidino-2-phenyl-3-butanone hydrochloride appeared to be as effective as borax in preventing fungus growth. At the end of the first week of storage, none of the oranges treated with 5 per cent 1-piperidino-2-phenyl-3-butanone hydrochloride or 8 per cent borax showed any evidence of fungus growth, while 26.6 per cent of the untreated oranges were visibly infected. By the fourth week, only 2 or 6.6 per cent of the oranges treated with 5 per cent 1-piperidino-2-phenyl-3-butanone hydrochloride revealed fungus growth, compared to 4 (13.3 per cent) obviously contaminated oranges treated with borax. At this time, however, 60 per cent of the controls had been noticeably attacked by fungi.

Experiment 2. As in the first experiment, 4 lots of 30 oranges each were again treated with alcoholic solutions of 1-piperidino-2-phenyl-3-butanone hydrochloride at concentrations of 0.5 per cent, 2 per cent, and 5 per cent and with an 8 per cent aqueous solution of borax, respectively. The stem-end of each orange was next inoculated, by means of an atomizer, with a spore suspension of *Phomopsis citri*. Thirty untreated oranges were similarly inoculated as controls. The various groups of oranges were stored in sterilized porcelain trays at room temperature and examined at weekly intervals.

Data given in table 2 indicate the percentages of sound oranges and of oranges manifesting fungus growth. Fungi of all types appearing (blue, green, white, and stem-end) were included in the enumeration of oranges grossly infected. After 7 days' storage, the number of sound oranges in the group treated with 5 per cent 1-piperidino-2-phenyl-3-butanone hydrochloride was 23 (76.6 per cent) compared to 21 (70 per cent) in the group sprayed with the borax solution, with 16 (53.3 per cent) sound oranges in the untreated or control group. At the end of the fourth week, 50 per cent of the oranges in each of the above treated groups were apparently sound, with 43.3 per cent sound in the control group. The incidence of oranges affected by fungus growth at final examination was 20 per cent of the lots treated respectively with 0.5 per cent and 2 per cent solutions of 1-piperidino-2-phenyl-3-butanone hydrochloride and 10 per cent of the group treated with the 5 per cent solution, with 26.6 per cent of the borax-treated group showing fungus growth, and 40 per cent of the oranges infected in the control group.

**DISCUSSION**

Various compounds have been tested for their effectiveness as mold-preventives for citrus fruits. Significant reduction in decay has been reported following application of sodium bicarbonate in concentrations of 2.5 to 3 per cent (Klotz, 1936). Inhibition of decay-producing organisms was observed with o-phenylphenol (Rattray, 1939) and diphenyl (MacIntosh, 1945), but not without adversely affecting the fruit. Childs and Siegler (1944) found thiourea in 5 per cent concentration effective in controlling stem-end rot and green mold decay, while Lauriol (1952), in a study of various methods to prevent citrus fruit spoilage, obtained best results with borate solutions. Soaking the fruit in 10 per cent sodium borate was more effective than 10 per cent metaborate, 10 per cent pentaborate, or 5 per cent borate.

From the results obtained in the present study, it is apparent that 1-piperidino-2-phenyl-3-butanone hydrochloride may be as effective as sodium borate in controlling fungus growth on oranges. The lower concentrations of the ketone required for comparable results seem to justify further work with this compound as a mold inhibitor for fruit.

**SUMMARY**

A ketone derivative, 1-piperidino-2-phenyl-3-butanone hydrochloride, possessing marked antifungal

### Table 1. Growth control of naturally occurring fungi of oranges (Experiment 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Per cent sound</th>
<th>Per cent with fungus growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days</td>
<td>Days</td>
</tr>
<tr>
<td>1-Piperidino-2-phenyl-3-butanone-HCl</td>
<td>%</td>
<td>7 14 21 28</td>
<td>7 14 21 28</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>76.6 67.6 67.3 66.6 63.3</td>
<td>3.3 3.3 10.0 13.3</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>63.3 56.6 66.5 65.3 66.6</td>
<td>10.0 13.3 32.0 33.0</td>
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<tr>
<td></td>
<td>5.0</td>
<td>93.3 86.6 76.6 66.3 3.3</td>
<td>0.0 3.3 3.3 6.6</td>
</tr>
<tr>
<td>Borax</td>
<td>8.0</td>
<td>93.3 76.6 73.3 66.6 66.6</td>
<td>0.0 3.3 10.0 13.3</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>53.3 50.0 50.0 50.0 50.0</td>
<td>26.6 30.0 50.0 50.0</td>
</tr>
</tbody>
</table>

### Table 2. Control of fungus growth on oranges following stem-end inoculation (Experiment 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Per cent sound</th>
<th>Per cent with fungus growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days</td>
<td>Days</td>
</tr>
<tr>
<td>1-Piperidino-2-phenyl-3-butanone-HCl</td>
<td>%</td>
<td>7 14 21 28</td>
<td>7 14 21 28</td>
</tr>
<tr>
<td></td>
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<td>6.6 6.6 10.0 20.0</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>83.3 76.6 73.3 66.6 66.6</td>
<td>3.3 3.3 3.3 32.0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>76.6 66.6 66.0 50.0 50.0</td>
<td>6.6 6.6 10.0 10.0</td>
</tr>
<tr>
<td>Borax</td>
<td>8.0</td>
<td>70.0 66.6 63.3 50.0 50.0</td>
<td>6.6 6.6 10.0 26.6</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>53.3 53.3 53.3 53.3 53.3</td>
<td>16.6 23.3 23.3 34.0</td>
</tr>
</tbody>
</table>

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properties and significant antibacterial action in vitro, has been tested as a preventive against fungus attack of fruit.

Definite experimental control of blue and green mold decay and stem-end rot of oranges was obtained following application of an alcoholic spray containing the fungicide in concentrations ranging from 0.5 to 5 per cent.

At 0.5 per cent, 1-piperidino-2-phenyl-3-butanone hydrochloride effected the same degree of protection as did an aqueous solution of borax at 8 per cent.

REFERENCES


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Isolation of Members of the Genus Salmonella by Membrane Filter Procedures

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The isolation of the microorganisms of the genus Salmonella is frequently difficult when their number is relatively small in comparison to the total bacterial population of the specimen. The use of selective agents in the medium may result in low recovery rates for Salmonella because many of these bacterial cells may die or be inhibited before they become adapted to the environmental changes. However, the use of a non-selective medium or one of low selectivity permits excessive growth of unwanted bacterial species which may suppress or overgrow the Salmonella colonies. Thus many of the procedures in current use may yield negative results when there is actually a small percentage of Salmonella present in the bacterial population.

This paper describes a procedure which appears to overcome some of the difficulties in the use of highly selective media and gives improved recovery rates for several species of Salmonella. The method consists of a short period of incubation in a selective enrichment liquid medium (Osborne and Stokes, 1955) which permits rapid multiplication of many Salmonella species with some reduction in growth of many other bacterial species. After incubation for approximately 6 hr in the enrichment medium, Salmonella are actively growing, having become adjusted to the selective medium while the other species, for the most part, are slowed in growth rate. The bacteria are then removed by filtration through a membrane filter and the membrane incubated on the single strength selective medium for the growth of colonies of Salmonella and further restriction of other bacterial species. By use of water samples which had been seeded with freshly isolated Salmonella, or with feces from mice fed cultures of Salmonella, improved recovery rates were demonstrated by this membrane filter (MF) technique.

METHODS AND MATERIALS

Media used in this investigation were selenite brilliant green broth (SBG) single and double strength, with or without the addition of sodium sulfapyridine 0.05 and 0.1 per cent, respectively. The formulas are shown in table 1.

The media are boiled for 5 to 10 min. Excessive heating or pressure sterilization is to be avoided. The pH of the finished medium is 7.2.

One ml of various dilutions selected to give an ade-