Antifungal Activity of Trimethyltinbenzoate-4-Picoline

M.K. Choudhury*, S.A. Ado†, A. Triny† and A.S. Shettima
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences.
†Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, PMB 1045, Nigeria
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Trimethyltinbenzoate-4-picoline was screened for its antifungal activities against six fungi consisting of
two moulds, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor and
two yeasts, Brettanomyces anomalum, Saccharomyces cerevisiae isolated from diseased grape plants
(Vitis vinifera). A. niger and A. versicolor were inhibited by the compound at a concentration of 0.5 mg/
ml, while A. flavus and S. cerevisiae required 1 mg/ml concentration. A. fumigatus and B. anomalum
required a higher concentration of 2.5 mg/ml for inhibition. The Minimum Inhibitory Concentration (MIC)
for A. versicolor, A. niger, A. flavus, S. cerevisiae, A. fumigatus and B. anomalum were found to be 0.4, 0.45,
0.8, 0.9, 2.5 and 2.5 mg/ml, respectively.

Despite the routine use of Chemical pesticides for
the control of fungal diseases of crop plants, these dis-
ese have become more severe in different parts of
the world. One of the possible explanations for this is
the development of tolerance by some pathogens. The sus-
pected microbial characteristics responsible for the re-
sistance are mutability of genes controlling the site of
action of the pesticides, the rate of multiplication of
the pathogens as well as the viability rate of resistant mu-
tants. These pesticides are environmental pollutants when
used in excess. The production of organotin compounds
is receiving more attention due to their wide range of
biological properties. The biocidal activities of organotin
carboxylates24 and triorganotin compounds5, the antifun-
gal activity of n-tributyltin acetate against some com-
mon yam (Dioscorea rotundata, staple food in Africa) rot
fungi6, the acute toxicity of triphenyltin acetate7, the
trypanocidal activity of bis (tri-n-butyltin) oxide against
Trypanosoma brucei8 and the antitumor activities of
organotin carboxylates9,12 have been reported earlier.

The introduction of organotin compounds has had
the effect of reducing long-term hazards, where the fact
that organotin agrochemicals eventually break down
physiochemically or biologically to harmless non-toxic
forms of tin when released to the environment13 is an
advantage. In this study, the antifungal activity of
trimethyltinbenzoate-4-picoline has been determined for
its potential fungicidal activities against fungal pathogen
of grapevine (Vitis vinifera) plants.

All chemicals were procured from BDH, England;
Potato Dextrose Agar and Nutrient Broth were supplied
by Oxoid Ltd., Basingstoke, Hants, England. Methanol
was distilled and then dried before use.

Trimethyltinbenzoate-4-picoline complex was pre-
pared by reacting trimethyltin chloride, sodium benzoate
and 4-picoline in equimolar proportion in dry methanol
at room temperature (25°C) under stirring for 12 h. The pre-
cipitated sodium chloride was filtered, the filtrate was
concentrated in vacuo and the product crystallized from
methanol, m.p. 76-78°(l).

\[
(CH_3)_3SnOCH_3 \quad (I)
\]

The IR (Nujol) spectrum of the compound (I) showed
bands at 1620 (strong, \(-O\cdot CO\cdot\)) and 1050 cm\(^{-1}\) (weak,

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Six fungi consisting of four moulds, Aspergillus flavius, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor and two yeasts, Brettanomyces anomala, Saccharomyces cerevisiae, that were isolated from diseased grape plants (Vitis vinifera) collected from Ahmadu Bello University campus, Zaria during winter time (December-January). The organisms were kept in Potato Dextrose Agar (PDA) slants under refrigerated condition.

The antifungal activity of the compound was determined using agar diffusion method. The medium was prepared as follows according to standard procedure. PDA (39 g) was dissolved in boiling water (100 ml), sterilised at 121° for 15 min, the solution poured onto different petridishes (20 ml each) and then allowed to solidify aseptically. The petridishes were of 200 mm diameter size.

Ten different concentrations in the range of 0.25 to 10 mg/ml were prepared by dissolving the compound in 10% dimethyl sulphoxide (DMSO in water). Each fungal isolate was streaked onto separate plate of freshly prepared sterile PDA using sterile cotton swabs. Six wells were punched on each inoculated PDA plate using sterilised No. 8 cork borer and properly labelled according to different concentrations of the compound. The wells were filled with 0.5 ml of different concentrations of the test compound with a control (10% DMSO) in each plate and then incubated at ambient temperature (29±1°) for 48-72 h. After incubation period the cultures were examined for inhibition growth which appeared as clear zones around the wells. The diameter of zones of inhibition were measured in mm (Table 1).

The Minimum Inhibitory Concentration (MIC) was determined using serial dilution method in test tubes containing nutrient broth. The medium was prepared according to specification. Nutrient broth (13 g) was dissolved in distilled water (1 litre), sterilised at 121° for 15 min and then distributed into the final container. The lowest concentration which showed inhibition in agar diffusion assay for each organism was serially diluted, each fungal isolate was inoculated into different tubes containing the compound at different concentrations and then incubated at ambient temperature (29±1°) for 48-72 h. After incubation period the tubes were examined for the growth of organisms. The highest dilution in the series for each organism showing no growth was considered to be the MIC of the test organism (Table 2).

All the organisms tested were inhibited by trimethyltinbenzoate-4-picoline and variations were observed as regards to the lowest concentration of the compound. For instance, A. niger and A. versicolor were inhibited when tested against a concentration of 0.5 mg/ml, whereas, A. flavus and S. cerevisiae were inhibited

<p>| TABLE 1: RESPONSES OF FUNGAL ISOLATES TO TRIMETHYL TinBENZOATE-4-PICOLINE |
|----------------------------------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Name of fungi</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. versicolor</td>
<td>15</td>
<td>32.5</td>
<td>35</td>
<td>37</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>A. niger</td>
<td>10.5</td>
<td>35.8</td>
<td>40.3</td>
<td>42.8</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>A. flavus</td>
<td>-</td>
<td>35.3</td>
<td>40</td>
<td>44.8</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>-</td>
<td>13.3</td>
<td>14.3</td>
<td>23.3</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17.8</td>
<td>21</td>
<td>23.8</td>
<td>25</td>
<td>29.5</td>
</tr>
<tr>
<td>B. anomala</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.8</td>
<td>14.3</td>
<td>16</td>
<td>16.8</td>
<td>19.3</td>
</tr>
</tbody>
</table>

10% DMSO (control) and 0.25 mg/ml concentration did not show any inhibition in all cases. - sign indicates no inhibition, while, NT indicates not tested.
TABLE 2: ANTIFUNGAL ACTIVITY OF TRIMETHYLTINBENZOATE-4-PICOLINE

<table>
<thead>
<tr>
<th>Name of fungi</th>
<th>Concentration in mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>+</td>
</tr>
<tr>
<td>A. niger</td>
<td>-</td>
</tr>
<tr>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>A. flavus</td>
<td>+</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>-</td>
</tr>
<tr>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>-</td>
</tr>
<tr>
<td>B. anomala</td>
<td>-</td>
</tr>
</tbody>
</table>

Minimum inhibitory concentrations of trimethyltinbenzoate-4-picoline in mg/ml (*) against various fungal isolates tested ‘+’ sign indicates inhibition and ‘-’ sign indicates no inhibition.

at a concentration of 1 mg/ml. In the case of A. fumigatus and B. anomala, a higher concentration of 2.5 mg/ml was required for inhibition. Increasing size in the diameter of zones of inhibition with increase of concentration was observed for all the isolates. The maximum concentration of 10 mg/ml was used in the case of A. fumigatus and B. anomala (Table 1).

In all cases, the MIC was either the same or lower than the lowest concentration that showed inhibition when the compound was tested using agar diffusion method. The MIC values for A. versicolor, A. niger, A. flavus and S. cerevisiae, A. fumigatus and B. anomala were found to be 0.4, 0.45, 0.8, 0.9, 2.5 and 2.5 mg/ml respectively.

The responses of the fungal isolates to trimethyltinbenzoate-4-picoline suggest that the compound has great potential as a chemical protectant against diseases likely to be caused by these organisms as only a low concentration of the chemical is required to inhibit any of the organisms tested. Organotin compounds break down to harmless non-toxic forms of tin after bio-degradation or physico-chemical reaction and therefore this compound is expected to have no threat to the environment when used as a crop protectant chemical. Further work needs to be carried out to determine the factors that affect the efficacy of the compound. This is because the efficacy of any potential crop protection chemical is known to vary with factors such as pH, temperature, aeration, the nature of disease agent and the size of inoculum. The results obtained in the study clearly show that the organotin compound tested has a great potential as a fungicide against fungal diseases of grapevine.

REFERENCES

1. Lewis, F.H., Plant Diseases, 1980, 64, 258.