

## Antifungal Activity of 4-Methyl-6-alkyl-2H-pyran-2-ones

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A number of 4-methyl-6-alkyl- $\alpha$ -pyrones were synthesized and characterized on the basis of  $^1\text{H}$  NMR and mass spectroscopy. These compounds were tested in vitro against pathogenic fungi, namely, *Sclerotium rolfsii* Saccardo, *Rhizoctonia bataticola* (Taub.) Butler, *Pythium aphanidermatum* (Edson) Fitz., *Macrophomina phaseolina* (Tassi), *Pythium debaryanum* (Hesse), and *Rhizoctonia solani* Nees. Lower homologues were less effective, whereas compounds such as 4-methyl-6-butyl- $\alpha$ -pyrone, 4-methyl-6-pentyl- $\alpha$ -pyrone, 4-methyl-6-hexyl- $\alpha$ -pyrone, and 4-methyl-6-heptyl- $\alpha$ -pyrone were found effective against all of the test fungi. They inhibited mycelial growth by approximately 50% ( $\text{ED}_{50}$ ) at 15–50  $\mu\text{g}/\text{mL}$ . 4-Methyl-6-hexyl- $\alpha$ -pyrone, which was found most effective, was tested against *S. rolfsii* in a greenhouse at 1, 5, and 10% concentrations. The 10% aqueous emulsion of 4-methyl-6-hexyl- $\alpha$ -pyrone suppressed disease development in tomato by 90–93% as compared with the untreated infested soil in the greenhouse after 35 days of treatment.

**KEYWORDS:** 4-Methyl-6-alkyl- $\alpha$ -pyrones; antifungal activity; *Sclerotium rolfsii*; *Rhizoctonia bataticola*; *Pythium aphanidermatum*; *Macrophomina phaseolina*; *Pythium debaryanum*; *Rhizoctonia solani*; green house testing

### INTRODUCTION

The yield of major food and cash crops is reduced nearly 20% by pathogenic fungi. Among these are *Sclerotium rolfsii* and *Rhizoctonia bataticola*, devastating soil borne fungi with a wide host range. They infect seeds, seedlings, and mature plants in the field causing collar rot, wilt, damping off, dry root rot, etc. A large number of chemical crop protectants used to control these organisms are detrimental to the environment and human health and need to be replaced by safe, biodegradable products.

Lactones are of considerable significance to the fragrance and food industries due to their potent fragrant and organoleptic characteristics, which may convey either desirable or objectionable qualities (1). It has been reported that 6-alkyl- $\alpha$ -pyrones show a smooth progressive change in flavor with increasing molecular weight. The lower homologues are coumarin-coconut, while the higher homologues are waxy, green, and floral. Of the 6-alkyl- $\alpha$ -pyrones evaluated, 6-pentyl- $\alpha$ -pyrone (6-PAP) was considered to have the most pleasant flavor (2). Besides its organoleptic properties, the antifungal activity of the natural product 6-PAP, a fungal metabolite isolated from various *Trichoderma* spp., is well-known and its in vitro activity against a range of phytopathogens has been reported (3–7). As a natural product, 6-PAP is innately biodegradable. Combined with its established use as a food additive and assuming low toxicity, 6-PAP has been found as an attractive candidate for development as an agricultural fungicide. Prior to its identification as a natural product, the chemical synthesis of 6-PAP and its structural analogues received attention; however, the synthesis of 6-PAP

has been found costly. Therefore, its cost is an apparent obstacle to further development. Earlier structure–activity relationships determining the antifungal activity of a range of synthetic analogues of 6-PAP indicated that structural requirements for antifungal activity appeared to be stringent. Shortening of the 6-alkyl substituents resulted in a marked loss of activity, as did saturation of the  $\Delta^2$ -bond of the pyrone ring (8). The 6-pent-1-enyl-substituted analogues showed similar antifungal activity. It was found that 4-methyl-6-pentyl-2H-pyran-2-one, a substituted analogue of 6-PAP, showed comparable activity in vitro and could be prepared with relative ease and economy relative to 6-PAP (9).

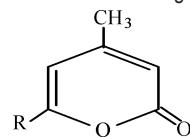
To further study the structure–activity relationship, several 4-methyl-6-alkyl- $\alpha$ -pyrones were prepared. In this paper, we report the in vitro and in vivo fungicidal activities of 4-methyl-6-alkyl-2H-pyran-2-ones against a number of soil borne pathogens.

### MATERIALS AND METHODS

**Chemicals and Reagents.** Aliphatic acids, thionyl chloride, aluminum chloride, and 3,3-dimethyl acrylic acid were procured locally. Thionyl chloride was distilled before use. The organic solvents used for syntheses were analytical grade and distilled and dried before use.

**Plant Pathogenic Fungi.** Plant pathogenic fungi such as *S. rolfsii* Saccardo, *R. bataticola* (Taub.) Butler, *Pythium aphanidermatum* (Edson) Fitz., *Macrophomina phaseolina* (Tassi), *Pythium debaryanum* (Hesse), and *Rhizoctonia solani* Nees were collected from the Indian Type Culture, Division of Plant Pathology, Indian Agricultural Research Institute (New Delhi, India). Pathogenic fungi were maintained on potato dextrose agar (PDA) at 25 °C and were subcultured on PDA Petri dishes for 5–6 days at 28 °C prior to use as inoculums.

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**Table 1.** Antifungal Activity of 4-Methyl-6-alkyl- $\alpha$ -pyrones against Soil Borne Pathogenic Fungi

compd no.	R	ED <sub>50</sub> ( $\mu\text{g mL}^{-1}$ ) <sup>a</sup> pathogenic fungi <sup>b</sup>					
		Sr	Rs	Rb	Mp	Pa	Pd
1	—CH <sub>3</sub>	712.63	492.98	1185.66	823.52	450.75	1140.96
2	—CH <sub>2</sub> CH <sub>3</sub>	665.30	690.40	625.53	267.49	407.62	580.33
3	—n(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	250.87	149.46	562.39	328.62	572.71	288.13
4	—i(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	313.37	437.17	403.49	1207.04	475.25	437.09
5	—n(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	77.99	50.08	212.34	235.02	227.19	142.79
6	—i(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	39.45	48.69	68.44	47.26	52.40	37.03
7	—n(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	22.45	34.49	34.16	36.77	39.12	20.56
8	—n(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	15.10	18.66	24.53	30.96	16.61	14.91
9	—n(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	30.7	45.04	47.04	82.15	74.64	71.71
10	—n(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>	121.84	115.90	132.36	163.89	123.78	174.64
11	—n(CH <sub>2</sub> ) <sub>8</sub> CH <sub>3</sub>	396.98	130.32	212.75	352.43	203.93	283.65
12	—n(CH <sub>2</sub> ) <sub>9</sub> CH <sub>3</sub>	562.21	267.34	649.00	548.37	287.12	594.38
13	—CH(C <sub>2</sub> H <sub>5</sub> )(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	349.00	183.66	303.13	261.94	276.96	150.97
14	—C(CH <sub>3</sub> )=CH <sub>2</sub>	180.01	205.99	373.88	337.54	337.06	188.84
15	—CH=CHCH <sub>3</sub>	403.82	293.51	360.99	276.02	102.18	95.49
16	—CICH <sub>2</sub>	46.93	22.75	111.23	72.08	121.94	101.99
17	—CH=C(C(CH <sub>3</sub> ) <sub>2</sub>	322.57	286.01	328.36	377.48	205.86	264.23
18	hexaconazole (standard)	18.34	12.96	4.36	10.53	8.47	12.30

<sup>a</sup> Average of five replicates. <sup>b</sup> Sr, *S. rolfsii*; Rs, *R. solani*; Rb, *R. bataticola*; Mp, *M. phaseolina*; Pa, *P. aphanidermatum*; and Pd, *P. debaryanum*.

**Chromatography and Spectroscopy.** Thin-layer chromatography (TLC) was performed on 250  $\mu\text{m}$  silica gel G plates preactivated at 120 °C for 2 h. The plates were developed in chloroform and visualized either by iodine vapor or spraying with an alcoholic solution of 2,4-dinitrophenyl hydrazine. Gas liquid chromatographic (GLC) analyses were performed on a Hewlett-Packard model 5890 A, gas liquid chromatograph fitted with a HP-17 capillary column (10 m  $\times$  0.53 mm i.d., 2.54  $\mu\text{m}$  film thickness) and equipped with a flame ionization detector and a Hewlett-Packard 3390A integrator. The operating conditions were as follows: The column temperature was programmed from 70 °C for 1 min to 250 °C at 10 °C min<sup>-1</sup> with injector temperatures of 300 °C. Nitrogen was used as a carrier gas with a flow rate of 20 mL min<sup>-1</sup>. Infrared (IR) spectra were recorded on a Nicolet (Impact 400) FT-IR spectrophotometer as neat. The <sup>1</sup>H NMR spectra were recorded on a Varian EM 360 L (60 MHz) and on a Bruker 300 AC (300 MHz) spectrometer using tetramethylsilane as the internal reference. Mass spectra were recorded on a HRGC-MEGA 2 series gas chromatograph coupled to a FISONS-TRIO 1000 ion trap mass spectrometer and connected with a Panasonic KX-P1150 multimode printer. The ionization potential was 70 eV. The gas chromatograph was fitted with a HP-17 capillary column (30 m  $\times$  0.25 mm i.d.; film thickness, 0.1–0.15  $\mu\text{m}$ ). Helium was used as a carrier gas at a flow rate of 2 mL min<sup>-1</sup>.

**General Procedure of Synthesis of 4-Methyl-6-alkyl-2H-pyran-2-ones.** 4-Methyl-6-alkyl-2H-pyran-2-ones were prepared from methyl-3-methyl-2-butenoate, which in turn was prepared from 3,3-dimethyl acrylic acid by esterification (10) using methanol and sulfuric acid and was purified by distillation (bp 130–131 °C; yield, 93–95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.2 (d, 6H,  $J$  = 10 Hz), 3.99 (s, 3H), 5.70 (s, 1H). Methyl-3-methyl-2-butenoate was converted to methyl-3-methyl-5-oxo-5-alkyl-2-pentenoates (keto esters) by Friedel–Crafts acylation using aluminum chloride and dichloromethane as the solvent in the presence of different acyl chlorides. The crude keto ester thus obtained was further purified by fractional distillation under reduced pressure. These keto esters were converted to 4-methyl-6-alkyl- $\alpha$ -pyrones by lactonization using a mixture of glacial acetic acid and sulfuric acid (9:1). The 4-methyl-6-alkyl- $\alpha$ -pyrones thus obtained were further purified from the crude products by fractional vacuum distillation at reduced pressure. The

identity of the product was confirmed by comparison of their NMR, IR, and gas chromatography–mass spectrometry reported in the literature (11).

**Antifungal Assay.** The antifungal activity was tested in vitro against *S. rolfsii*, *R. solani*, *R. bataticola*, *M. phaseolina*, *P. aphanidermatum*, and *P. debaryanum* and in vivo on tomato seedlings infested with *S. rolfsii*.

**In Vitro Antifungal Activity.** The above-synthesized compounds were tested for their ability to inhibit above soil borne pathogenic fungi against the standard fungicide hexaconazole. The concentrations of the latter were those recommended by the manufacturer. The fungicidal activity of synthesized compounds was evaluated at various concentrations by the poisoned food technique using PDA media. The ready-made PDA medium (39 g) was suspended in distilled water (1000 mL) and heated to boiling until completely dissolved. The medium and Petri dishes were autoclaved at 120 °C for 30 min. These compounds were tested at concentrations of 250, 125, 100, 50, 25, and 10  $\mu\text{g/mL}$ . A stock solution of 1000  $\mu\text{g/mL}$  was prepared, which was further diluted with acetone to give the required concentrations. Acetone (1 mL) was used as the control. These solutions were added to the media (65 mL) contained in conical flasks to obtain the desired concentrations of the test compounds in the media. The medium was poured into a set of two Petri dishes (90 cm in diameter) under aseptic conditions in a laminar flow hood. The plates were kept under UV light in the laminar flow chamber for solidification of the media. After solidification, a 5 mm  $\times$  5 mm mycelial plug cut from the actively growing front of a 2 week old colony of the desired pathogenic fungus was then placed with the inoculum side down in the center of each treatment plate, aseptically. Treated Petri dishes were then incubated at 28 °C till the fungal growth was almost complete in the control plates. All experiments were in quadruplet for each treatment against each fungus.

**Recording of Observations.** The mycelial growth of fungus (cm) in both treated (T) and control (C) Petri dishes was measured diametrically. The mean and standard errors were calculated from the four replicates of each treatment, and the percentage inhibition of growth (*I*) was calculated using the following formula

$$\text{corrected inhibition } (\%) = \frac{C - T}{C} \times 100$$

**Calculation of ED<sub>50</sub> Values.** For calculation of ED<sub>50</sub> values (effective dose required for 50% inhibition of growth), the percent inhibition was converted to corrected percent inhibition by using Abbott's formula:

$$\text{corrected inhibition } (\%) = \frac{\% I - CF}{100 - CF} \times 100$$

where CF is the correction factor obtained by the equation

$$\text{correction factor (CF)} = \frac{9 - C}{C} \times 100$$

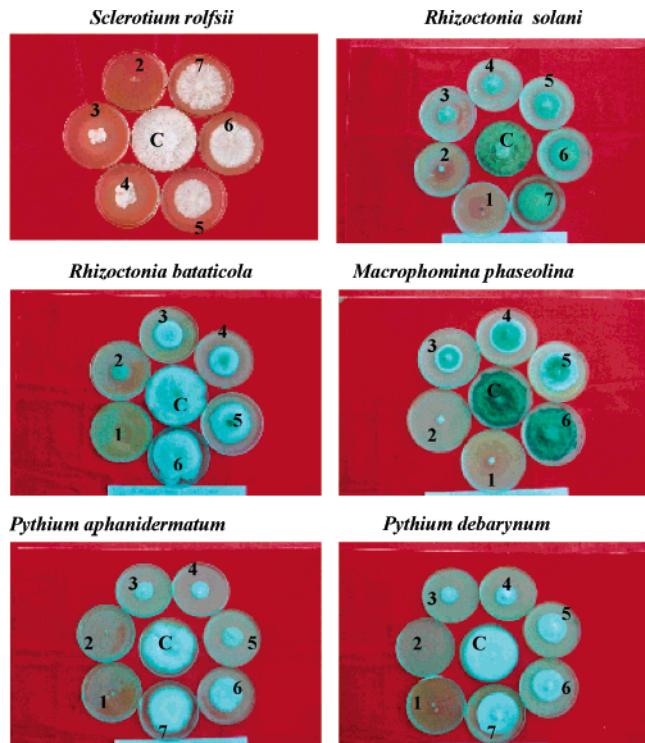
where 9 is the diameter of the Petri dish in cm and C is the diameter of growth of the fungus in control plates. From the concentration (ppm) and corresponding corrected percentage inhibition data of each compound, the ED<sub>50</sub> (ppm) value was calculated statistically by Probit analysis with the help of Probit package of MSTATC software using a personal computer. ED<sub>50</sub> values were calculated (effective dose for 50% inhibition  $\mu\text{g mL}^{-1}$ ) for inhibition of growth using the Basic LD<sub>50</sub> program version 1.1.

**In Vivo Antifungal Activity.** The most active compound of the series was tested in vivo for antifungal activity against *S. rolfsii* in pots. *S. rolfsii* was chosen because it has an extensive host range; at least 500 species in 100 families are susceptible. It primarily attacks host stems, although it may infect any part of a plant under favorable environmental conditions including roots, fruits, petioles, leaves, and flowers. The first signs of infection, although usually undetectable, are dark-brown lesions on the stem at or just beneath the soil level; the first visible symptoms are progressive yellowing and wilting of the leaves. Following this, the fungus produces abundant white, fluffy mycelia on infected tissues and soil. Seedlings are very susceptible and die quickly once they become infected. Older plants that have formed woody tissue are gradually girdled by lesions and eventually die. Invaded tissues are pale brown and soft but not watery.

**Inoculum Preparation.** Mycelial mats of *S. rolfsii* were grown in potato dextrose broth in 250 mL conical flasks and incubated horizontally at 27 °C. After 7 days, the medium was decanted and 200 mL of sterile deionized water was added to each bottle and incubated vertically at 20 °C for 3–4 weeks. Chlamydospores were harvested by rinsing the cultures two or three times with deionized water and homogenizing in a small blender for 1 min. The mycelial suspension then was ground in a glass tissue grinder to further break up the mycelia. The suspension was filtered through two layers of cheesecloth and sonicated in a water bath for two 30 s periods to disrupt any remaining viable mycelia. Microscopic examination verified that no cytoplasm remained in the mycelial fragments. Inoculum from 10 flasks was thoroughly incorporated into 2.5 kg of composted soil previously sifted through a 2 mm sieve and sterilized by autoclave at 120 °C at 15 psi for 15 min. The infested soil was incubated in the laboratory at room temperature (approximately 25 °C) for 5–7 days. Sterilized, autoclaved, uninfested soil was used as a control in all experiments.

**Treatments.** The experimental treatments for the disease control experiment in the greenhouse were (i) uninfected soil, moistened with water as a check; (ii) infested soil, only water added; (iii) infested and moistened soil treated with the formulated compound (1, 5, and 10% concentration); and (iv) infested and moistened soil treated with the commercial fungicide metalaxyl (0.364, 0.728, and 1.46 mL a.i. per 150 cm<sup>3</sup> of the soil).

**Disease Control in Greenhouse.** After a 5–7 day incubation period, the infested soil was treated by incorporating 84 mL of 1, 5, and 10% aqueous emulsion of formulated compound into 2.5 kg of soil at the rate of 5.0 mL of aqueous emulsion in 150 cm<sup>3</sup> of the soil. The treated soil was placed in double polyethylene bags that were then closed tightly and incubated for 7 days. Metalaxyl was incorporated similarly at 0.364, 0.728, and 1.46 mL a.i. per 150 cm<sup>3</sup> of the soil as above. After the incubation period, soil from each treatment was placed in 20 6.6 cm diameter standard plastic pots, and one 4–6 week old tomato seedling was transplanted into the soil in each pot. Pots were placed randomly



**Figure 1.** Effect of different concentrations of 6-hexyl-4-methyl-2H-pyran-2-ones on the mycelial growth of test fungi (250, 125, 100, 50, 25, and 10  $\mu\text{g/mL}$ ). Key ( $\mu\text{g/mL}$ ): 1, 250; 2, 125; 3, 100; 4, 50; 5, 25; 6, 10; 7, 6.25; and C, control.

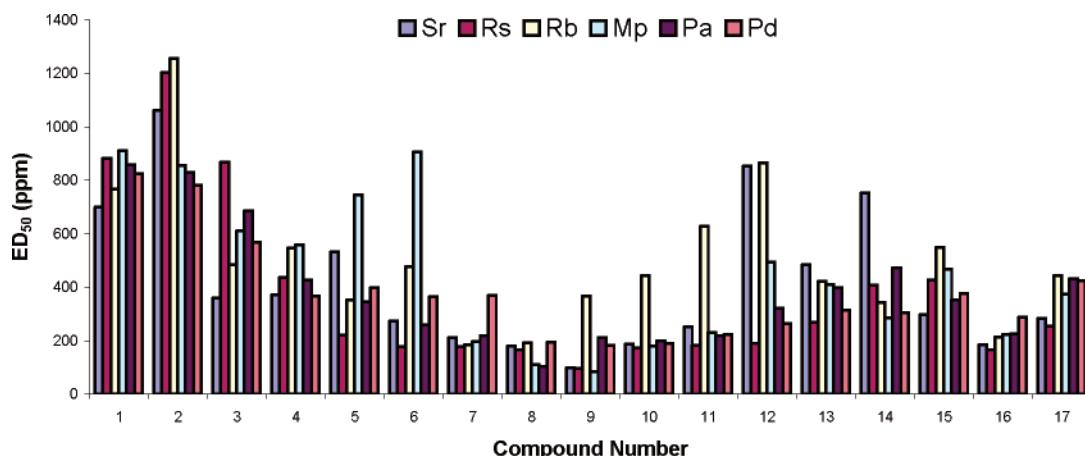
on the greenhouse bench. Disease characteristics (wilting) and mortality were assessed 7, 14, 21, 28, and 35 days after transplanting and weekly thereafter. The number of symptomless plants was recorded for each treatment at each assay date and expressed in terms of the proportion of symptomless plants. Six trials of the experiment with all concentrations were repeated. Disease severity was rated daily after inoculation based on a scale from 0 to 5 as follows: 0 for no visible disease symptoms; 1 for slightly wilted leaves, with brownish lesions beginning to appear on the stems; 2 for 30–50% of the entire plant diseased; 3 for 50–70% of the entire plant diseased; 4 for 70–90% of the entire plant diseased; and 5 for a dead plant. Data are the means of 20 plants per treatment.

**Analysis.** Data from the above experiments in the greenhouse were transformed as the arcsine of the square root of the proportion of the symptomless plant stand. It was analyzed as repeated measure design and analysis of variance determined using MSTATC software (Statistical Package). The significance level was determined before analysis based on the observed variation in plant growth among trials due to external greenhouse variables.

## RESULTS AND DISCUSSION

A number of 4-methyl-6-alkyl-2H-pyran-2-ones were prepared by the lactonization of methyl-3-methyl-5-oxo-5-alkyl-2-pentenoates or ethyl-3-methyl-5-oxo-5-alkyl-2-pentenoates using a suitable concentrated acid (conveniently a mixture of glacial acetic acid and concentrated sulfuric acid). They were purified from the crude products by fractional vacuum distillation (yield, 68–78%). Boiling points are similar to those reported in the literature (11). The purity of these compounds was further confirmed by TLC and GLC. The FT-IR spectra of 4-methyl-6-alkyl-2H-pyran-2-ones showed absorption at 1720–1740 (C=O) and 1630–1640 and 1550–1560  $\text{cm}^{-1}$  (C=C), characteristic of  $\alpha$ -pyrone.

The <sup>1</sup>H NMR spectra of 4-methyl-6-substituted-2H-pyran-2-ones showed ring protons as singlets at  $\delta$  5.80 and  $\delta$  5.90 (one



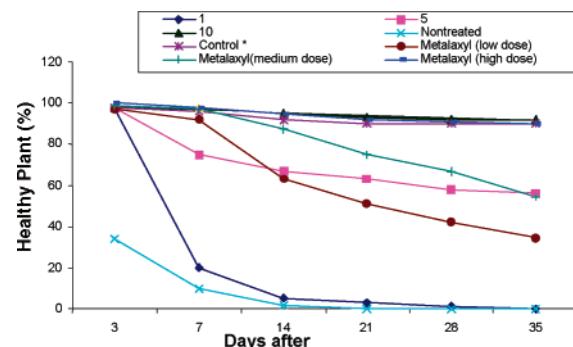
**Figure 2.** Comparison of antifungal activity of different analogues of 4-methyl-6-alkyl- $\alpha$ -pyrones against Sr, *S. rolfsii*; Rs, *R. solani*; Rb, *R. bataticola*; Mp, *M. phaseolina*; Pa, *P. aphanidermatum*; and Pd, *P. deberianum*.

proton each). They also indicated a normal aliphatic chain and three vinyl protons typical of an ABX spin system. The mass spectra of 4-methyl-6-alkyl-2H-pyran-2-ones showed, besides the molecular ion peak, various fragment ion peaks. The mass spectra showed major ions at  $m/z$  95 and 109 arising from  $\alpha$  cleavage of the C-6 alkyl group with or without the loss of the C<sub>4</sub> methyl group, respectively, and  $m/z$  53 ( $C_4H_5^+$ ) and  $m/z$  27 ( $C_2H_3^+$ ).

**Antifungal Activity.** Substituted 4-methyl-6-alkyl-2H-pyran-2-ones were tested for fungicidal activity against above-reported five soil borne pathogenic fungi by the poisoned food technique. A smooth progressive increase in fungicidal activity was observed with increasing molecular weight (Table 1). The lower homologues such as 6-methyl-, 6-ethyl-, and 6-propyl-substituted compounds resulted in a marked loss of activity (Table 1), while compounds, namely, 6-butyl-4-methyl-2H-pyran-2-one, 6-isobutyl-4-methyl-2H-pyran-2-one, 6-pentyl-4-methyl-2H-pyran-2-one, 6-hexyl-4-methyl-2H-pyran-2-one, and 6-heptyl-4-methyl-2H-pyran-2-one, were found to be very effective against all of the tested fungi (Table 1). Compounds with 6-octyl, 6-nonyl, and 6-decanoyl side chains showed a marked decrease in activity (Table 1). The 6-hexyl-4-methyl-2H-pyran-2-one showed maximum activity against *S. rolfsii* ( $ED_{50} = 15.10 \mu\text{g mL}^{-1}$ ), *P. debaryanum* ( $ED_{50} = 14.91 \mu\text{g mL}^{-1}$ ), and *P. aphanidermatum* ( $ED_{50} = 16.61 \mu\text{g mL}^{-1}$ ) (Table 1). Figure 1 shows the inhibition of mycelial growth at different concentrations (250, 125, 100, 50, 25, 10, and 6.25  $\mu\text{g/mL}$ ) as compared to control by 6-hexyl-4-methyl-2H-pyran-2-one, when tested in vitro. A complete inhibition of mycelial growths of the different pathogenic fungi was observed at 250 and 150  $\mu\text{g/mL}$  concentrations (Figure 1) as compared to control (full growth).

Within this and similar length conditions, compounds with a branch(es), unsaturation, or heteroatom(s) were prepared to examine additional effects. Branching at the  $\beta$ -position to the pyrone ring was found to enhance activity; that is, the activity of 6-isopropyl-4-methyl-2H-pyran-2-one (compound 4,  $ED_{50} = 313.37 \mu\text{g/mL}$ , Table 1) was considerably higher than that of 6-ethyl-4-methyl-2H-pyran-2-one (compound 2,  $ED_{50} = 665.30 \mu\text{g/mL}$ ) with the corresponding length, and similarly, 6-isobutyl-4-methyl-2H-pyran-2-one (compound 6,  $ED_{50} = 39.45 \mu\text{g/mL}$ ) had a significantly higher activity than 6-propyl-4-methyl-2H-pyran-2-one (compound 3,  $ED_{50} = 250.87 \mu\text{g/mL}$ ) against *S. rolfsii*.

The unsaturated 6-alkyl substituents (compounds 14, 15, and 17) resulted in loss of fungicidal activity. The 6-chloromethyl-4-methyl- $\alpha$ -pyrone (compound 16) was found more active than



**Figure 3.** Percent healthy tomato plants after treatment of *S. rolfsii* infested soil with 6-hexyl-4-methyl- $\alpha$ -pyron and metalaxyl. Each point represents the mean of repeated trials of the experiment with 20 replications (one plant per pot) per trial. Soil was treated with 1, 5, and 10% aqueous emulsion of the formulated compound and with metalaxyl at 0.364, 0.728, and 1.46 mL a.i. per  $150 \text{ cm}^3$  of the soil, respectively. \*Control, uninfested soil.

6-methyl and 6-ethyl analogues with the corresponding chain length. The antifungal activity of 6-alkyl-4-methyl-2H-pyran-2-one followed the order methyl < ethyl < propyl < butyl < pentyl < hexyl < heptyl < octyl > nonyl in straight chain compounds (Table 1 and Figure 2).

**Disease Control in the Greenhouse.** Among the compounds tested for antifungal activity in vitro, 6-hexyl-4-methyl- $\alpha$ -pyrone (compound 8 in Table 1 and Figure 1) was found to be most effective against all of the test pathogenic fungi. Therefore, it was thought worthwhile to study the antifungal activity of 6-hexyl-4-methyl- $\alpha$ -pyrone against *S. rolfsii* in the greenhouse for control in tomatoes (*Lycopersicon esculentum* Mill.) at different concentrations (5.0 mL of 1, 5, and 10% aqueous emulsion in  $150 \text{ cm}^3$  of the soil). Metalaxyl, a commercial fungicide, was used for comparison.

The occurrence of symptoms in different treatments was recorded at each assay date and expressed in terms of the proportion of symptomless (healthy) plant stands. The results are shown in Figure 3.

The untreated control resulted in 100% disease incidence (0% healthy plant stands) 35 days after transplanting. The treatment of soil with 1% aqueous emulsion of the compound resulted in only 20% healthy plant stands after 7 days of transplanting, whereas no healthy plant stands were observed after 35 days of transplanting. Soil treated with metalaxyl at 0.364 mL a.i. per  $150 \text{ cm}^3$  of the soil resulted in 34.3% healthy plant stands 35

days after transplanting; however, because of experimental variations, the healthy plant stands were not significant ( $P = 0.103$ ).

The treatment of soil with 5% aqueous emulsion of the compound showed survival of more than 60% healthy plant stands after 35 days of transplanting. However, the healthy plant stands were not significantly ( $P > 0.1$ ) different from the soil treated with a medium dose of metalaxyl (at 0.728 a.i. per 150 cm<sup>3</sup>; 60% healthy plant stands, **Figure 3**).

The treatment of soil with 10% aqueous emulsion of the compound resulted in significantly ( $P < 0.1$ ) greater healthy plant stands (92.4%) than that obtained with metalaxyl (90.3%) 35 days after transplanting (**Figure 3**). Thus, significant differences existed among concentration and time for disease control experiments in the greenhouse. The 10% aqueous emulsion of the compound prevented tomato damping off significantly. It allowed a plant stand comparable to the uninfested control and metalaxyl (at 0.728 and 1.46 mL a.i. per 150 cm<sup>3</sup> of the soil).

In conclusion, among 4-methyl-6-alkyl- $\alpha$ -pyrones, 6-butyl-, 6-pentyl-, 6-hexyl-, and 6-heptl-substituted 4-methyl pyrones showed antifungal activity against *S. rolfsii* Saccardo, *R. bataticola* (Taub.) Butler, *P. aphanidermatum* (Edson) Fitz., *M. phaseolina*, *P. debaryanum*, and *R. solani* Nees in vitro. The 4-methyl-6-hexyl- $\alpha$ -pyrone, which was found to be the most effective of the series, reduced disease development on tomato caused by *S. rolfsii*. Experimental conditions were optimized for survival of *S. rolfsii* in soil and disease development in the greenhouse. Despite favorable conditions for the pathogens, the aqueous formulation of 4-methyl-6-hexyl- $\alpha$ -pyrone reduced the pathogen population and resulted in higher healthy plant stands as compared to the control (pathogen only).

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