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ARTICLE

Antifungal Activity of 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-Alkyl Ethers

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A number of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-alkyl ethers were prepared, separated into their *E* and *Z* isomers, and characterized on the basis of ^{1}H NMR and mass spectroscopy. These compounds were tested in vitro for antifungal activity against four important phytopathogenic fungi, namely, *Sclerotium rolfsii*, *Rhizoctonia bataticola*, *Macrophomina phaseolina*, and *Sclerotinia sclerotiorum*. *E* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-propyl ether (ED_{50} = 32.36 μg mL^{-1}) and 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-(1′′-methyl) ethyl ether (ED_{50} = 35.50 μg mL^{-1}) showed maximum antifungal activity against *R. bataticola* and *S. rolfsii*, respectively, whereas 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether was found to be active against *M. phaseolina* (ED_{50} = 31.08 μg mL^{-1}) and *S. sclerotiorum* (ED_{50} 21.39 μg mL^{-1}), respectively. The *Z* isomer of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-pentyl ether, which was found to be most effective, was tested against *S. sclerotiorum* in a greenhouse at 1 and 5% concentrations. The 5% aqueous emulsion of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether suppressed disease development in pea by 90−95% as compared with the untreated infested soil in the greenhouse after 21 days of treatment.

KEYWORDS: α-Ionone; antifungal; oxime ethers; *Rhizoctonia bataticola*; *Macrophomina phaseolina*; *Sclerotium rolfsii*; *Sclerotinia sclerotiorum*

INTRODUCTION

The terpenoids obtained from nature are a rich source of bioactive molecules. They are considered as a bountiful domain because of their versatility in bioactivity (*1*), target specificity, environmentally benign nature, and nonpersistency. The essential oils, which are the primary source of terpenoids, have been found to possess antifeedant (*2*), repellent (*3*), insect growth regulatory (*4*), feeding deterrent, fungicidal fumigant (*5*), antifungal (*6*), and antibacterial (*7*) activities.

A variety of terpenoid juvenile hormone (JH) analogues (*8*) have been invented, and the majority of them possess sesquiterpenoid structure (*9*). With the progress of research on JH analogues, the oxime ether group of the compounds has become the center of attraction for their insect growth regulatory activity (*10*, *11*). It has been also found that unlike the natural product as such, their derivatives are more stable in the environment. A few strobilurin fungicides containing an oxime ether moiety, namely, kresoxim-methyl (*12*) and trifloxystrobin (*13*), have been commercialized to control powdery mildew of various fruit crops, which indicates the potential of this group for more investigation for controlling plant pathogenic fungi.

α-Ionone is a cyclic monoterpenoid ketone naturally obtained from the oil of orris (*Iris florentina*) root. As it is from natural sources it can be considered as an effective substrate for the synthesis of desired oxime ethers. In the present study, we report the synthesis and in vitro and in vivo antifungal activities of a number of oxime ethers, prepared from α-ionone and separated into their *E* and *Z* isomers, against four widely occurring soilborne plant pathogenic fungi, namely, *Rhizoctonia bataticola* (Taub) Butler, the causal organism for damping-off of seedlings; *Macrophomina phaseolina* (Tassi) Goid, responsible for charcoal rot of soybean, peanut, and corn, stem rot of jute; *Sclerotium rolfsii* (Sacc.) Curzi, which causes southern blight of cucurbitaceous crops; and *Sclerotinia sclerotiorum*, which causes water-soaked spots of irregular shape on fruits, stems, and leaves of beans, cabbage, carrots, cucumbers, lettuce, onions, peas, pumpkins, squash, and tomatoes.

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MATERIALS AND METHODS

Chemicals and Reagents. α-Ionone ($C_{13}H_{20}O$, MW 192.30), obtained from Florentine iris (*I. florentina*) was used as lead molecule for the synthesis of oxime ethers and was procured from Fluka Chemika. Alkyl halides, sodium hydride, and hydroxylamine hydrochloride were procured locally and used directly without further purification. All of the solvents used in synthesis as well as in chromatography were distilled and dried before use.

Plant Pathogenic Fungi. Plant pathogenic fungi such as *R. bataticola* (Taub) Butler, *M. phaseolina* (Tassi) Goid., *S. rolfsii* (Sacc.) Curzi, and *S. sclerotiorum* 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.

Chromatography and Spectroscopy. Thin layer chromatography (TLC) was performed on 20 cm × 20 cm glass plates coated with 0.5 mm silica gel G, containing 10% of gypsum as binder, air-dried, and

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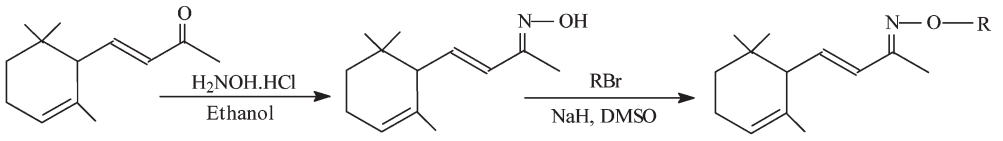
Scheme 1. Scheme for the Synthesis of 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N-O*-Alkyl Ethers



4'-(2,6,6-trimethyl -2-cyclohexen-1-yl) -3'-butene-2'-one

4'-(2,6,6-trimethyl -2-cyclohexen-1-yl) -3'-butene-2'-ketoxime

4'-(2,6,6-trimethyl-2-cyclohexen -1-yl)-3'-butene-2'-ketoxime-N-O -alkyl ether

R = CH_3, C_2H_5, C_3H_7, $CH(CH_3)_2$, C_4H_9, $CH_2CH(CH_3)_2$, C_5H_{11}, $C_2H_4CH(CH_3)_2$, C_6H_{13}, C_7H_{15}, C_8H_{17} and $C_{10}H_{21}$.

preactivated at 120 °C for 2 h before use. Hexane and acetone in the ratios of 9:1 and 8:2 were used as developing solvent, and iodine vapor was used as visualizing agent. *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N-O*-alkyl ethers were separated using a glass column (75 cm × 2 cm i.d.) packed with preactivated silica gel (50 g, 60−120 mesh) in hexane and eluted with a mixture of hexane and chloroform in various ratios. Different fractions (25 mL) were collected and distilled on a water bath. The fractions containing similar compounds were combined and purified further for characterization. A gas−liquid chromatograph (GLC) (Hewlett-Packard 5890 series II) equipped with a flame ionization detector, HP-1 megabore column (10 m × 0.53 mm i.d. and 2.65 μm film thickness) and HP 3390A integrator was used to determine the ratio of *E* and *Z* isomers in the reaction mixture. The operating conditions were oven, injector, and detector temperatures at 150, 250, and 250 °C, respectively. Nitrogen was used as carrier at a flow rate of 20 mL min^{-1}. The proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian EM 360 L (60 MHz) and on a Bruker 400 AC (400 MHz) instrument. The solvents used were carbon tetrachloride (CCl_4) and deuteriochloroform ($CDCl_3$) containing tetramethylsilane ($(CH_3)_4Si$, TMS) as the internal standard. The chemical shifts are expressed in δ values (ppm), and coupling constants (*J*) are given in hertz (Hz). 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 × 0.25 mm i.d.; film thickness, 0.1−0.15 μm). Helium was used as a carrier gas at a flow rate of 2 mL min^{-1}.

General Procedure for the Synthesis of 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime. Hydroxylamine hydrochloride (0.75 g, 0.011 mol) was added to a stirred solution of α-ionone (1.9 g, 0.01 mol) dissolved in dry and distilled ethyl alcohol (25 mL) (**Scheme 1**). Stirring was continued for another 2 h with simultaneous monitoring by TLC (solvent system hexane/acetone 80:20, R_f = 0.33). After completion of the reaction, the reaction mixture was poured into water and extracted with diethyl ether (3 × 25 mL). The solvent extracts were combined and dried over anhydrous sodium sulfate. The ether was completely distilled off. An amber-colored viscous liquid product was obtained and used for further reaction.

General Procedure for the Synthesis of 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N-O*-Alkyl Ether. Dimethyl sulfoxide (dried over molecular sieve, 5 mL) was slowly added with stirring to sodium hydride (0.024 g, 0.01 mol, weighed in a drybox) in an atmosphere of dry nitrogen. Nitrogen was dried by passing though a train consisting of (a) a trap, (b) a wash bottle containing concentrated sulfuric acid, and (c) a drying tube containing fresh soda lime. Stirring was further continued for about 30 min until the evolution of hydrogen ceased. Into the above stirred solution was added dropwise 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime (0.01 mol), maintaining the temperature at 5 °C (ice bath). The reaction mixture was stirred for another 1 h, which was followed by the addition of the appropriate alkyl halide (0.011 mol), and the mixture was again stirred for another 2 h (**Scheme 1**). The progress of the reaction was monitored by TLC (solvent system hexane/acetone, 9:1, v/v) and iodine as visualizing agent. After completion of the reaction, the reaction mixture was poured into ice-cold water and extracted with ethyl acetate (3 × 25 mL). The ethyl acetate layer was separated, washed with water, dried over anhydrous sodium sulfate, and concentrated in vacuum, which gave an amber-colored viscous oily liquid. The oily residue was separated on a silica gel column with hexane and hexane/chloroform in different ratios as eluting solvent separating *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N-O*-alkyl ether. The physicochemical properties are given in **Table 1**.

Antifungal Activity. The antifungal activity was tested in vitro against *R. bataticola* (Taub) Butler, *M. phaseolina* (Tassi) Goid., *S. rolfsii* (Sacc.) Curzi, and *S. sclerotiorum*.

In Vitro Antifungal Activity. The above-synthesized compounds were tested for their ability to inhibit the above soilborne pathogenic fungi. The fungicidal activity of synthesized compounds was evaluated at various concentrations by the poisoned food technique using PDA media (*14*). 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 then tested at concentrations of 1000, 500, 250, 125, 100, 50, 25, and 10 μg/mL. A stock solution of 1000 μg/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 (9 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 mycelial disk 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 until the fungal growth was almost complete in the control plates. All experiments were in quadruplicate 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:

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

Calculation of ED_{50} Values. For the calculation of ED_{50} values (effective dose required for 50% inhibition of growth), the percent inhibition was converted to corrected percent inhibition by using Abbott's formula

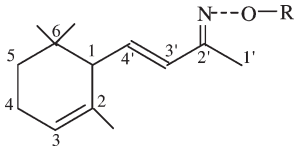
$$\text{corrected inhibition } (\%) = \frac{\%I-\text{CF}}{100-\text{CF}} \times 100$$

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 (μg mL^{-1}) and corresponding corrected percentage inhibition data of each compound, Probit analysis was done with the help of the Probit package

Table 1. Physicochemical Properties of 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-Alkyl Ether



compd	R	name	physical state	molecular formula	TLC (R_f)[a]
1a	CH_3	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-methyl ether	liquid	$C_{14}H_{23}NO$	0.59
1b	CH_3	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-methyl ether	liquid	$C_{14}H_{23}NO$	0.34
2a	C_2H_5	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-ethyl ether	liquid	$C_{15}H_{25}NO$	0.64
2b	C_2H_5	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-ethyl ether	liquid	$C_{15}H_{25}NO$	0.36
3a	C_3H_7	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-propyl ether	liquid	$C_{16}H_{27}NO$	0.71
3b	C_3H_7	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-propyl ether	liquid	$C_{16}H_{27}NO$	0.38
4a	$CH(CH_3)_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-(1′′-methyl)ethyl ether	liquid	$C_{16}H_{27}NO$	0.72
4b	$CH(CH_3)_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-(1′′-methyl)ethyl ether	liquid	$C_{16}H_{27}NO$	0.39
5a	$CH_2CH{=}CH_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime-*N*-*O*-2′′propenyl ether	liquid	$C_{16}H_{25}NO$	0.74
5b	$CH_2CH{=}CH_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-2′′-propenyl ether	liquid	$C_{16}H_{25}NO$	0.42
6a	C_4H_9	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-butyl ether	liquid	$C_{17}H_{29}NO$	0.76
6b	C_4H_9	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-butyl ether	liquid	$C_{17}H_{29}NO$	0.42
7a	$CH_2CH(CH_3)_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-(2′′-methyl)propyl ether	liquid	$C_{17}H_{29}NO$	0.75
7b	$CH_2CH(CH_3)_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-(2′′-methyl)propyl ether	liquid	$C_{17}H_{29}NO$	0.42
8a	C_5H_{11}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-pentyl ether	liquid	$C_{18}H_{31}NO$	0.79
8b	C_5H_{11}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether	liquid	$C_{18}H_{31}NO$	0.44
9a	$C_2H_4CH(CH_3)_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-(3′′-methyl)butyl ether	liquid	$C_{18}H_{31}NO$	0.77
9b	$C_2H_4CH(CH_3)_2$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-(3′′-methyl)butyl ether	liquid	$C_{18}H_{31}NO$	0.42
10a	C_6H_{13}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-hexyl ether	liquid	$C_{19}H_{33}NO$	0.79
10b	C_6H_{13}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-hexyl ether	liquid	$C_{19}H_{33}NO$	0.46
11a	C_7H_{15}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-heptyl ether	liquid	$C_{20}H_{35}NO$	0.80
11b	C_7H_{15}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-heptyl ether	liquid	$C_{20}H_{35}NO$	0.49
12a	C_8H_{17}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-octyl ether	liquid	$C_{21}H_{37}NO$	0.81
12b	C_8H_{17}	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-octyl ether	liquid	$C_{21}H_{37}NO$	0.51
13a	$C_{10}H_{21}$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-decyl ether	liquid	$C_{23}H_{41}NO$	0.84
13b	$C_{10}H_{21}$	4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-decyl ether	liquid	$C_{23}H_{41}NO$	0.55

[a] Solvent system: hexane/acetone (9:1).

of MSTATC software using a personal computer. ED_{50} values were statistically calculated (*15*) for the inhibition of growth using the Basic LD_{50} program version 1.1.

In Vivo Antifungal Activity. The most active compound of the series, 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether (ED_{50} = 31.39 $\mu g\,mL^{-1}$), was tested in vivo for antifungal activity against *S. sclerotiorum* in pots keeping carbendazim as standard fungicide for comparison. The concentrations of the latter were those recommended by the manufacturer. *S. sclerotiorum* was chosen because it is among the most nonspecific, omnivorous, and successful of plant pathogens with an extensive host range; at least 361 species in 64 families are susceptible. The first signs of infection are water-soaked spots on the stem at or just beneath the soil level. The symptoms are water-soaked spots on fruits, stems, leaves, and petioles, which usually have an irregular shape. These spots enlarge, and a cottony mycelium covers the affected area. The fungus spreads, and the plant becomes a soft, slimy, water-soaked mass. Seedlings are very susceptible and die quickly once they become infected. In contrast to the water-soaked symptoms, the older plants may exhibit "dry" lesions on the stalk, stems, or branches. The lesions enlarge, girdling the plant part to yellowish brown in color, causing it to die.

Inoculum Preparation. Mycelial mats of *S. sclerotiorum* 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. Ascospores or sclerotia 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 twice for 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, with only water added; (iii) infested and moistened soil treated with the formulated compound (1 and 5% concentration); and (iv) infested and moistened soil treated with the commercial fungicide carbendazim (0.364 and 0.728 mL of ai/150 cm^3 of soil).

Disease Control in the Greenhouse. After a 5−7 day incubation period, the infested soil was treated by incorporating 84 mL of 1 and 5% aqueous emulsion of formulated compound into 2.5 kg of soil at the rate of 5.0 mL of aqueous emulsion in 150 cm^3 of the soil. The treated soil was placed in double polyethylene bags that were then closed tightly and incubated for 7 days. Carbendazim was incorporated similarly at 0.364 and 0.728 mL of ai/150 cm^3 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 pea seedling was transplanted into the soil in each pot. Pots were placed randomly on the greenhouse bench. Disease characteristics (soft, slimy, water-soaked spots) and mortality were assessed 7, 14, and 21 days after transplantation 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 on the basis of a scale from 0 to 5 as follows: 0 for no visible disease symptoms; 1 for water-soaked spots on stems and leaves, with cottony mycelium 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 a repeated measure design and analysis of variance determined using MSTATC software (Statistical Package). The significance level was determined before analysis on the basis of the observed variation in plant growth among trials due to external greenhouse variables.

RESULTS AND DISCUSSION

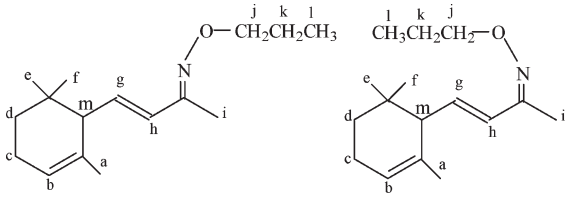
Synthesis. 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime required for the preparation of oxime ethers was obtained by reacting 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-one (α-ionone) with hydroxylamine hydrochloride. The oximes are usually a 1:1 mixture of cis and trans isomers and are inseparable by conventional chromatographic technique. 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-alkyl ethers were synthesized by reacting 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime with appropriate alkyl halide (**Scheme 1**). These oxime ethers being a mixture of two isomers were separated and purified on a silica gel column eluting with hexane and hexane/chloroform (90:10) as eluting solvent. Although it was expected that the *E* and *Z* isomers would be produced in equal amounts, actually in the reaction mixture the *E* isomer of oxime ether was found to be the predominant product and the *E*/*Z* ratio calculated by GLC was found to be 2:1. An unequivocal assignment of their stereochemistry was accomplished with ^{1}H NMR and mass spectroscopy (**Table 2**).

The ^{1}H NMR spectra of all the pure compounds (**Table 2**) exhibited a similar general pattern in a homologous series with some distinct differences that were confirmatory enough to identify each of the *E* and *Z* isomers. For example, α-ionone oxime on reaction with propyl bromide produced 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-propyl ether as an amber-colored viscous liquid. The oily residue was separated into *E* and *Z* isomers on a silica gel column eluting with *n*-hexane and gave the 3*E* isomer, whereas elution with hexane/chloroform (9:1) gave the 3*Z* isomer. Formation of oxime ether was characterized by the appearance of a two proton triplets at δ 4.08−4.12 due to O−C**H$_2$** (**j**) with a coupling constant (*J*) value of 3 Hz (**Table 2**). The product 3*E* showed two three-proton singlets at δ 0.94 and 0.92 due to C**H$_3$** (**e**) and C**H$_3$** (**f**), whereas in product 3*Z* isomer one six-proton singlet was found downfield at δ 1.26 for [C**H$_3$** (**e**) and C**H$_3$** (**f**)]. Similarly, one proton doublet was found at δ 6.10 due to =C**H** (**h**) in the 3*E* isomer, but was found downfield at δ 6.75 in the case of the 3*Z* isomer. The difference in the chemical shifts in CH_3 protons in the 3*E* and 3*Z* isomers is because of the difference in anisotropic shielding in the *E* and *Z* isomers with their fixed geometries. The presence of protons due to C**H$_3$** (**e**), C**H$_3$** (**f**) and =C**H** (**h**) upfield in product 3*E* isomer as compared to the 3*Z* isomer indicated that in the 3*E* isomer the *O*-propyl group is *syn* to the methyl group (**i**), whereas in the 3*Z* isomer it is *anti* to the methyl group (**i**). Thus, on the basis of ^{1}H NMR spectra, the product 3*E* was assigned the *E* configuration and product 3*Z* as the *Z* configuration.

The mass spectra of products 3*E* and 3*Z* showed a molecular ion peak at *m*/*z* 249 with relative abundances of 89 and 7%, respectively. The higher relative abundance of the molecular ion peak indicates greater stability of 3*E*, which can be correlated with the 2:1 ratio of 3*E* and 3*Z* in the reaction mixture. As both the products showed the same molecular ion peak, these two products are geometric isomers. A distinct difference in the fragmentation pattern as well as in percent relative abundance with respect to base peak has been observed in products 3*E* and 3*Z*. The mass spectrum of product 3*E* showed a base peak at *m*/*z* 134 (100%) along with fragment ion peaks with higher relative abundance at *m*/*z* 249 and 193 than the other isomer. In contrast, 3*Z* has shown a base peak at *m*/*z* 83 (100%) formed by retro-Diels−Alder reaction of the unsaturated ring. All other sets of isomers have shown similar trends of mass fragmentation for *E* and *Z* isomers. Both products showed similar fragment ion peaks but with different relative abundance percentages at *m*/*z* 234 ($M^+ - CH_3$), 207 ($M^+ - C_3H_6$), 206 ($M^+ - C_3H_7$), 193 ($M^+ - CH_3 - C_3H_5$), 178 ($M^+ - 2CH_3 - C_3H_6$), 160 ($M^+ - C_3H_7 - NOH - CH_3$), 150 ($M^+ - C_5H_9NO$), 136 ($M^+ - C_5H_9NO - CH_2$), 134 ($M^+ - 2CH_3 - C_3H_6 - CNOH_2$), 119 ($M^+ - NO - C_4H_9$), 117 ($M^+ - NO - C_4H_{11}$), 107 ($M^+ - NO - C_5H_9$), 105 ($M^+ - NO - C_4H_9 - CH_2$), 93 ($M^+ - C_5H_9NO - C_4H_9$), 91 ($M^+ - 2CH_3 - C_3H_6 - NO - C_4H_9$ or $M^+ - NO - C_4H_9 - 2CH_2$), 85 ($M^+ - CH_3 - C_3H_5 - C_8H_{12}$), 83 ($M^+ - C_3H_6 - C_9H_{16}$), 79 ($M^+ - C_5H_9NO - C_4H_9 - CH_2$), and 77 ($M^+ - 2CH_3 - C_3H_6 - NO - C_4H_9 - CH_2$), but their percent relative abundances were different. Thus, on the basis of ^{1}H NMR and mass spectroscopy, products 3*E* and 3*Z* were characterized as 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-propyl ether and 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-propyl ether.

Antifungal Activity. *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-alkyl ethers were tested for fungicidal activity against the above-reported four soilborne pathogenic fungi by the poisoned food technique. A smooth progressive increase in fungicidal activity was observed with increasing molecular weight (**Table 3**). The lower homologues such as *O*-methyl- and *O*-ethyl-substituted compounds resulted in a marked loss of activity (**Table 3**), whereas the *E* isomer of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-propyl ether was found to be more effective against *R. bataticola* (ED_{50} = 32.36 μg mL^{-1}) as compared to its *Z* isomer (ED_{50} = 156.85 μg mL^{-1}). It was also found to be effective against other test fungi as compared to methyl and ethyl derivatives. The *E* isomer of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-isopropyl ether was found to be effective against *S. rolfsii* (ED_{50} = 35.50 μg mL^{-1}) as compared to its *Z* isomer (ED_{50} = 129.03 μg mL^{-1}). Here a pronounced effect of branching of the side chain on the antifungal activity was observed in the case the *E* isomer, where a 3-fold increase in activity was observed when the *n*-propyl group (ED_{50} = 101.30 μg mL^{-1}) was replaced by and isopropyl group (ED_{50} = 35.50 μg mL^{-1}). On the other hand, 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether has been found to exhibit highest activity against *M. phaseolina* (ED_{50} = 31.08 μg mL^{-1}) and *S. sclerotiorum* (ED_{50} = 21.39 μg mL^{-1}), respectively. There was an increase in antifungal activity with an increase in the number of carbon atoms to a certain extent. Compounds, namely, *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-propyl ether, *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-isopropyl ether, *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-butyl ether, *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-isobutyl ether, and *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-pentyl ether, were found to be very effective against all of the tested fungi (**Table 3**). Both *E* and *Z* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O* with isopentyl, hexyl, heptyl, octyl, and decyl ether showed a marked decrease in activity (**Table 3**). The antifungal activity of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime

Table 2. Spectral Data of 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*-*O*-Alkyl Ether



3E **3Z**

compd	R	^{1}H NMR (δ)	mass (*m*/*z*)
1a	CH_3	6.04 (1H, h, d, *J* = 8 Hz), 5.81 (1H, g, dd, *J* = 3 Hz), 5.43 (1H, b, s), 3.84 (2H, j, s), 2.23 (1H, m, d, *J* = 3 Hz), 1.94 (3H, i, s), 1.71(1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, *J* = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s)	221 (M^+, 89%), 203 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
1b	CH_3	6.57 (1H, h, d, *J* = 8 Hz), 5.79 (1H, g, dd, *J* = 3 Hz), 5.46 (1H, b, s), 3.87 (2H, j, s), 2.00 (1H, m, d, *J* = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, *J* = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s)	221 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
2a	C_2H_5	6.11 (1H, h, d, *J* = 8 Hz), 5.85 (1H, g, dd, *J* = 3 Hz), 5.42 (1H, b, s), 4.1 (2H, j, q, *J* = 3 Hz), 2.23 (1H, m, d, *J* = 3 Hz), 2.01 (3H, l, t, *J* = 3 Hz), 1.94 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, *J* = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s)	235 (M^+, 89%), 217 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
2b	C_2H_5	6.67 (1H, h, d, *J* = 8 Hz), 5.83 (1H, g, dd, *J* = 3 Hz), 5.4 (1H, b, s), 4.08 (2H, j, q, *J* = 3 Hz), 2.06 (3H, l, t, *J* = 3 Hz), 2.00 (1H, m, d, *J* = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, *J* = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s)	235 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
3a	C_3H_7	6.10 (1H, h, d, *J* = 8 Hz), 5.83 (1H, g, dd, *J* = 3 Hz), 5.43 (1H, b, s), 4.12 (2H, j, t, *J* = 3 Hz), 2.23 (1H, m, d, *J* = 3 Hz), 1.94 (3H, i, s), 2.02 (2H, k, m), 1.71(1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, *J* = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (3H, l, t, *J* = 3 Hz)	249 (M^+, 89%), 234 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
3b	C_3H_7	6.75 (1H, h, d, *J* = 8 Hz), 5.90 (1H, g, dd, *J* = 3 Hz), 5.45 (1H, b, s), 4.08 (2H, j, t, *J* = 3 Hz), 2.01 (2H, k, m), 2.00 (1H, m, d, *J* = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, *J* = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (3H, l, t, *J* = 3 Hz)	249 (M^+, 7%), 207(2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
4a	$CH(CH_3)_2$	6.19 (1H, h, d, *J* = 8 Hz), 5.83 (1H, g, dd, *J* = 3 Hz), 5.43 (1H, b, s), 4.23 (2H, j, h, *J* = 3 Hz), 2.23 (1H, m, d, *J* = 3 Hz), 2.02 (6H, l, d, *J* = 3 Hz), 1.94 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58(2H, d, t, *J* = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, *J* = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s)	249 (M^+, 89%), 234 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
4b	$CH(CH_3)_2$	6.83 (1H, h, d, *J* = 8 Hz), 5.90 (1H, g, dd, *J* = 3 Hz), 5.45 (1H, b, s), 4.26 (2H, j, h, *J* = 3 Hz), 2.03 (6H, l, d, *J* = 3 Hz), 2.00 (1H, m, d, *J* = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, *J* = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s)	249 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
5a	$CH_2CH{=}CH_2$	6.12 (1H, h, d, *J* = 8 Hz), 6.03 (1H, k, m), 5.81 (1H, g, dd, *J* = 3 Hz), 5.42 (1H, b, s), 5.23 (2H, l, m), 4.12 (2H, j, t, *J* = 3 Hz), 2.23 (1H, m, d, *J* = 3 Hz), 1.94 (3H, i, s), 1.71(1H, c_2, q, *J* = 3 Hz), 1.58(2H, d, t, *J* = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, *J* = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s)	247 (M^+, 89%), 232 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
5b	$CH_2CH{=}CH_2$	6.75 (1H, h, d, *J* = 8 Hz), 6.0 (1H, k, m), 5.90 (1H, g, dd, *J* = 3 Hz), 5.45 (1H, b, s), 5.32 (2H, l, m), 4.08 (2H, j, t, *J* = 3 Hz), 2.00 (1H, m, d, *J* = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, *J* = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s)	247 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
6a	C_4H_9	6.16 (1H, h, d, *J* = 8 Hz), 5.84 (1H, g, dd, *J* = 3 Hz), 5.4 (1H, b, s), 4.0 (2H, j, t, *J* = 3 Hz), 2.23 (1H, m, d, *J* = 3 Hz), 1.94 (3H, i, s), 2.02 (4H, k, m), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, *J* = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (3H, l, t, *J* = 3 Hz)	263 (M^+, 89%), 248 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
6b	C_4H_9	6.83 (1H, h, d, *J* = 8 Hz), 5.68 (1H, g, dd, *J* = 3 Hz), 5.37 (1H, b, s), 4.12 (2H, j, t, *J* = 3 Hz), 2.01 (4H, k, m), 2.00 (1H, m, d, *J* = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, *J* = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (3H, l, t, *J* = 3 Hz)	263 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
7a	$CH_2CH(CH_3)_2$	6.10 (1H, h, d, *J* = 8 Hz), 5.87 (1H, g, dd, *J* = 3 Hz), 5.4 (1H, b, s), 3.8 (2H, j, d, *J* = 3 Hz), 2.23 (1H, m, d, *J* = 3 Hz), 1.94 (3H, i, s), 2.02 (1H, k, m), 1.71 (1H, c_2, q, *J* = 3 Hz), 1.58 (2H, d, t, *J* = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, *J* = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (6H, l, d, *J* = 3 Hz)	263 (M^+, 89%), 248 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)

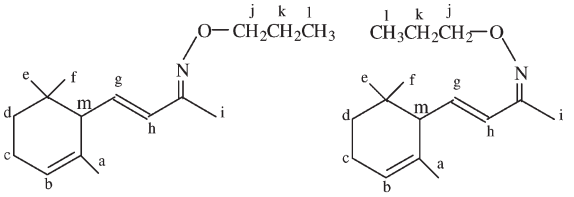
Table 2. Continued

compd	R	^{1}H NMR (δ)	mass (m/z)
7b	$CH_2CH(CH_3)_2$	6.8 (1H, h, d, J = 8 Hz), 5.90 (1H, g, dd, J = 3 Hz), 5.45 (1H, b, s), 3.84 (2H, j, d, J = 3 Hz), 2.01 (1H, k, m), 2.00 (1H, m, d, J = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, J = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (6H, l, d, J = 3 Hz)	263 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
8a	C_5H_{11}	6.23 (1H, h, d, J = 8 Hz), 5.84 (1H, g, dd, J = 3 Hz), 5.42 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.23 (1H, m, d, J = 3 Hz), 1.94 (3H, i, s), 2.02 (6H, k, m), 1.71(1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.57 (3H, a, s), 1.42(1H, c_1, q, J = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (3H, l, t, J = 3 Hz)	277 (M^+, 89%), 262 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
8b	C_5H_{11}	6.96 (1H, h, d, J = 8 Hz), 5.72 (1H, g, dd, J = 3 Hz), 5.39 (1H, b, s), 4.08 (2H, j, t, J = 3 Hz), 2.01 (6H, k, m), 2.00 (1H, m, d, J = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, J = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (3H, l, t, J = 3 Hz)	277 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
9a	$C_2H_4CH(CH_3)_2$	6.23 (1H, h, d, J = 8 Hz), 5.84 (1H, g, dd, J = 3 Hz), 5.42 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.23 (1H, m, d, J = 3 Hz), 1.94 (3H, i, s), 2.02 (3H, k, m), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, J = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (6H, l, d, J = 3 Hz)	277 (M^+, 89%), 262 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
9b	$C_2H_4CH(CH_3)_2$	6.96 (1H, h, d, J = 8 Hz), 5.72 (1H, g, dd, J = 3 Hz), 5.39 (1H, b, s), 4.08 (2H, j, t, J = 3 Hz), 2.01 (3H, k, m), 2.00 (1H, m, d, J = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, J = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (6H, l, d, J = 3 Hz)	277 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
10a	C_6H_{13}	6.13 (1H, h, d, J = 8 Hz), 5.78 (1H, g, dd, J = 3 Hz), 5.43 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.23 (1H, m, d, J = 3 Hz), 1.94 (3H, i, s), 2.02 (8H, k, m), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, J = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (3H, l, t, J = 3 Hz)	291 (M^+, 89%), 276 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
10b	C_6H_{13}	6.65 (1H, h, d, J = 8 Hz), 5.90 (1H, g, dd, J = 3 Hz), 5.45 (1H, b, s), 4.08 (2H, j, t, J = 3 Hz), 2.01 (8H, k, m), 2.00 (1H, m, d, J = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, J = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (3H, l, t, J = 3 Hz)	291 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
11a	C_7H_{15}	6.10 (1H, h, d, J = 8 Hz), 5.87 (1H, g, dd, J = 3 Hz), 5.43 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.23 (1H, m, d, J = 3 Hz), 1.94 (3H, i, s), 2.02 (10H, k, m), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, J = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (3H, l, t, J = 3 Hz)	305 (M^+, 89%), 290 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
11b	C_7H_{15}	6.81 (1H, h, d, J = 8 Hz), 5.90 (1H, g, dd, J = 3 Hz), 5.4 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.01 (10H, k, m), 2.00 (1H, m, d, J = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, J = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (3H, l, t, J = 3 Hz)	305 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
12a	C_8H_{17}	6.16 (1H, h, d, J = 8 Hz), 5.80 (1H, g, dd, J = 3 Hz), 5.43 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.23 (1H, m, d, J = 3 Hz), 1.94 (3H, i, s), 2.02 (12H, k, m), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, J = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (3H, l, t, J = 3 Hz)	319 (M^+, 89%), 304 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
12b	C_8H_{17}	6.75 (1H, h, d, J = 8 Hz), 5.73 (1H, g, dd, J = 3 Hz), 5.4 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.01 (12H, k, m), 2.00 (1H, m, d, J = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, J = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (3H, l, t, J = 3 Hz)	319 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)
13a	$C_{10}H_{21}$	6.13 (1H, h, d, J = 8 Hz), 5.87 (1H, g, dd, J = 3 Hz), 5.5 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.23 (1H, m, d, J = 3 Hz), 1.94 (3H, i, s), 2.02 (16H, k, m), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.57 (3H, a, s), 1.42 (1H, c_1, q, J = 3 Hz), 0.94 (3H, e, s), 0.92 (3H, f, s), 0.90 (3H, l, t, J = 3 Hz)	347 (M^+, 89%), 332 (17%), 207 (4%), 206 (5%), 193 (89%), 178 (48%), 160 (8%), 150 (49%), 136 (28%), 134 (100%), 119 (22%), 117 (13%), 107 (35%), 105 (20%), 93 (83%), 91 (60%), 85 (7%), 83 (12%), 79 (25%), 77 (29%)
13b	$C_{10}H_{21}$	7.0 (1H, h, d, J = 8 Hz), 5.8 (1H, g, dd, J = 3 Hz), 5.45 (1H, b, s), 4.0 (2H, j, t, J = 3 Hz), 2.01 (16H, k, m), 2.00 (1H, m, d, J = 3 Hz), 1.97 (3H, i, s), 1.71 (1H, c_2, q, J = 3 Hz), 1.58 (2H, d, t, J = 3 Hz), 1.58 (3H, a, s), 1.45 (1H, c_1, q, J = 3 Hz), 1.26 (3H, e, s), 1.26 (3H, f, s), 0.91 (3H, l, t, J = 3 Hz)	347 (M^+, 7%), 207 (2%), 193 (5%), 178 (4%), 150 (5%), 136 (3%), 134 (10%), 119 (5%), 117 (4%), 107 (5%), 105 (3%), 93 (17%), 91 (14%), 85 (92%), 83 (100%), 79 (5%), 77 (7%)

N-O alkyl ethers followed the order methyl < ethyl < propyl < butyl < pentyl > hexyl > heptyl > octyl > decyl in straight-chain compounds (**Table 3**).

Disease Control in the Greenhouse. Among the compounds tested for antifungal activity in vitro, 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N-O*-pentyl ether

Table 3. Fungi Toxicity of 4′-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-ketoxime *N*−*O*-Alkyl Ethers against *Rhizoctonia bataticola*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, and *Sclerotinia sclerotiorum*



3E **3Z**

		R. bataticola ED_{50}(μg mL^{-1})		*M. phaseolina* ED_{50}(μg mL^{-1})		*S. rolfsii* ED_{50}(μg mL^{-1})		*S. sclerotiorum* ED_{50}(μg mL^{-1})	
compd	R	*E*	*Z*	*E*	*Z*	*E*	*Z*	*E*	*Z*
1	CH_3	387.93	412.86	306.54	310.93	237.61	258.52	441.74	516.87
2	C_2H_5	392.90	245.99	731.62	393.99	410.19	414.89	294.53	261.42
3	C_3H_7	32.36	156.85	181.26	235.76	101.30	132.22	257.20	219.70
4	$CH(CH_3)_2$	56.57	116.35	126.01	197.99	35.50	129.03	181.79	190.49
5	C_4H_9	67.89	113.63	100.64	173.89	113.23	88.89	107.39	163.66
6	$CH_2CH(CH_3)_2$	50.60	58.69	102.96	100.91	45.22	47.62	94.07	79.05
7	C_5H_{11}	39.17	57.56	94.75	31.08	43.56	41.65	32.68	21.39
8	$C_2H_4CH(CH_3)_2$	722.57	125.34	665.58	625.23	257.30	366.53	273.48	375.13
9	C_6H_{13}	894.89	326.69	750.68	962.75	572.80	912.11	427.72	243.85
10	C_7H_{15}	918.55	566.24	717.34	1091.25	974.61	956.26	392.18	419.18
11	C_8H_{17}	1094.84	1613.71	824.24	1109.20	1037.26	1356.83	837.26	584.24
12	$C_{10}H_{21}$	1943.64	2058.44	956.86	1759.29	842.46	1107.14	681.27	477.48

(compound **7** in **Table 3**) was found to be most effective against most of the test pathogenic fungi. Therefore, it was thought worthwhile to study the antifungal activity of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether against *S. sclerotiorum* (ED_{50} = 21.39 μg mL^{-1}) in the greenhouse for control in pea at different concentrations (5.0 mL of 1 and 5% aqueous emulsion in 150 cm^3 of soil). Carbendazim, 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 1**. The untreated control (pathogen only) resulted in 100% disease incidence (0% healthy plant stands) 21 days after transplantation. The treatment of soil with a 1% aqueous emulsion of the compound resulted in 65% healthy plant stands at 7 days after transplantation, whereas only 40% healthy plant stands were observed at 21 days after transplantation. Soil treated with carbendazim at 0.364 mL of ai/150 cm^3 of soil resulted in 80% healthy plant stands after 14 days and 75% at 21 days after transplantation. The treatment of soil with 5% aqueous emulsion of the compound resulted in significantly ($P < 0.1$) greater healthy plant stands. It showed survival of >90% healthy plant stands at 21 days after transplantation. However, the healthy plant stands were not significantly ($P > 0.1$) different from the soil treated with carbendazim (0.728 mL of ai/150 cm^3) (**Figure 1**). Thus, significant differences existed among concentration and time for disease control experiments in the greenhouse. The 5% aqueous emulsion of the compound prevented water-soaked spots in pea significantly. It allowed a plant stand comparable to the uninfested control and carbendazim (at 0.728 mL of ai/150 cm^3 of soil).

In conclusion, *E* isomers of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*E*)-ketoxime *N*-*O*-propyl ether (ED_{50} = 32.36 μg mL^{-1}) and 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′-(*E*)-ketoxime *N*-*O*-(1″-methyl)ethyl ether (ED_{50} = 35.50 μg mL^{-1}) showed maximum antifungal activity against *R. bataticola* and *S. rolfsii*, respectively, whereas 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether was found to be active against *M. phaseolina* (ED_{50} = 31.08 μg mL^{-1}) and *S. sclerotiorum* (ED_{50} = 21.39 μg mL^{-1}), respectively. Experimental conditions were optimized for survival of *S. sclerotiorum* in soil and disease development in the greenhouse. Despite favorable conditions for the pathogens, the 5% aqueous formulation of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether reduced the pathogen population and resulted in higher healthy plant stands as compared to nontreatment (pathogen only).

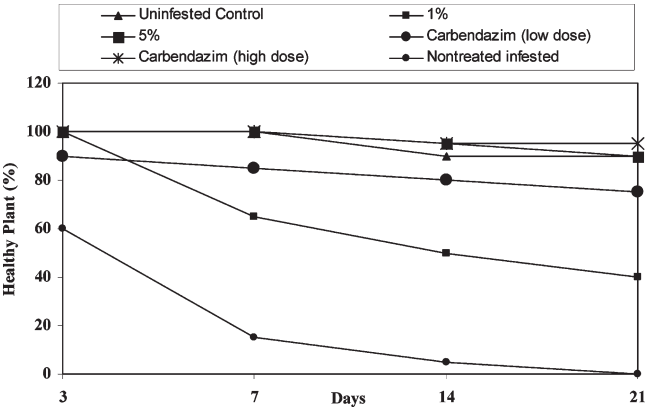


Figure 1. Percent healthy pea plants after treatment of *S. sclerotiorum* infested soil aqueous emulsion of 4′-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3′-butene-2′(*Z*)-ketoxime *N*-*O*-pentyl ether and carbendazim . Each point represents the mean of repeated trials of the experiment with 20 replications (one plant per pot) per trial. Soil was treated with 1 and 5% aqueous emulsion of the formulated compound and with carbendazim at 0.364 and 0.728 mL of ai/150 cm^3 of soil, respectively.

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