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Suppression of powdery mildew (*Sphaerotheca fuliginea*) in cucumber by the detergent Zohar LQ-215

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Abstract

The effect of the detergent Zohar LQ-215 alone or combined with the fungicide fenarimol (Rubigan) on cucumber powdery mildew (caused by *Sphaerotheca fuliginea*) was examined under controlled environmental conditions and in the field. In laboratory tests conducted on leaf disks, the efficacy of fenarimol was 30 times greater than that of Zohar LQ-215 as indicated by the ED_{50} values of the compounds (0.012% and 0.356%, respectively). Zohar LQ-215 did not reduce spore germination or germ-tube elongation, but inhibited the later stages of the disease cycle (i.e., mycelial growth and sporulation). Although the detergent was degraded relatively quickly, its effect on the polycyclic development of the disease lasted for up to 24 days after spraying. Low disease severity (2.4% compared with 27% in the control) was maintained following three applications of the detergent at 5 days intervals. In a greenhouse trial, a mixture of Zohar LQ-215 and fenarimol at half rate did not improve disease suppression beyond the effect of fenarimol applied alone at half rate. However, the effects of the two compounds were additive when applied in the field as a mixture.

Introduction

Powdery mildew caused by *Sphaerotheca fuliginea* (Schlechtend, Fr.) Pollaci, limits the production of cucurbits throughout the world [Sitterly, 1978]. Fungicide application is the major method used for disease management [Khadar and Abdou, 1972; Paulus *et al.*, 1976; 1978] but it does not always provide adequate disease suppression. Decreased sensitivity of the fungus to several groups of fungicides has been reported from cucurbit growing areas where fungicides have been used extensively for several seasons [Paulus *et al.*, 1976; Delp, 1988]. Moreover, the increasing concern for the environment and public health and the expanding competition in the agricultural market motivate growers to optimize disease management by reducing fungicide application. Planting cultivars resistant to powdery mildew is an alternative that can lead to reduction of fungicide use. However, this approach may provide only a partial solution for the problem because resistance is not always available. For example, a few melon (*Cucumis melo* L.) cultivars have race-specific resistance to powdery mildew but all the commercially important squash (*C. pepo*) and watermelon (*Citrullus vulgaris*) cultivars and most of the cucumber cultivars grown in Israel are susceptible to *S. fuliginea* [Cohen *et al.*, 1993; Cohen, unpublished].

Another possible alternative to fungicidal control is the use of non-toxic compounds. Several coating polymers, such as antitranspirants, mineral oils and surfactants have been used as artificial barriers on leaf surfaces and they inhibited the development of foliar pathogens on various host plants, including powdery mildew in cucurbits. Application of bicarbonate salts has been found to be effective [Elad *et al.*, 1989; Horst *et al.*, 1992; Ziv and Hagiladi, 1993; Ziv and Zitter, 1992]. Biological control is another non-

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chemical alternative for disease suppression. Several microorganisms have been found effective against various powdery mildew fungi. The most common is the hyperparasite *Ampelomyces quisqualis* (Sztejnberg *et al.*, 1989). Other studies have reported that soapy water had some preventive effect on powdery mildew on greenhouse - grown cucumber [Qvarnstron, 1989; Rasmussen *et al.*, 1991]. Zohar LQ-215 is a new detergent manufactured by Zohar Dalia of Israel. This compound acts directly on certain development stages of white fly (*Bemisia tabacii*) and on the transmission of the viruses ZYMV and CVYV in melons [Dubitzki *et al.*, 1991]. In field trials where the efficacy of Zohar LQ-215 against white fly was evaluated, it was observed that the severity of powdery mildew on plants treated with the detergent was lower than that on untreated plants. However, data were not properly recorded and analyzed. In the present study, the efficacy of Zohar LQ-215 in the suppression of powdery mildew on cucumbers was examined in laboratory, greenhouse and field trials. A preliminary report of part of the results has been published [Cohen *et al.*, 1994].

Materials and methods

Laboratory and greenhouse trials

In all experiments, a powdery mildew-susceptible cucumber culitvar, 'Bet-Alfa' (HaZera, Israel) was used. Seeds were sown in 250-ml plastic pots (one seed per pot), filled with vermiculite and peat (1:1 v/v). Pots were placed in a growth chamber with day length ranging from 10.5 to 12 h; the day temperature was maintained at 25 ± 5 °C and the night temperature at 15 ± 3 °C. Lighting was provided by high-pressure sodium vapour bulbs (300 μE m^{-2} sec^{-1}).

A culture from a single spore of *S. fuliginea* race 1, isolated from muskmelon (*C. melo*, cv. 'Ananas Yoqne'am') was used for inoculation in all trials. The culture was maintained on plants of *C. melo* cv. 'Ananas Yoqne'am' grown as above in a separate growth chamber. Cucumber plants were inoculated at the three-leaf stage by blowing air from infected plants towards the test plants. Although this inoculation procedure is not quantitative, relatively uniform disease developed on the inoculated plants. The severity of powdery mildew symptoms was evaluated visually. The adaxial surfaces of inoculated leaves were inspected after symptom appearance (on day 7), and disease severity (i.e., percentage leaf area with disease symptoms) was assessed with the aid of a disease assessment scale [Sitterly, 1978]. Assessments were carried out at 5 to 7-day intervals for 18–21 days.

Chemicals under test were applied to run-off by means of a hand sprayer. The detergent Zohar LQ-215 (170 g l^{-1} detergent, Zohar Dalia, Detergent factory, Daliya 18-920, Israel) and the fungicide fenarimol (Rubigan, 12% a.i. EC, DowElanco, King's Lynn, UK) were used. Application rates and timing are specified below. All greenhouse trials were laid out in a completely randomized design with five replicate pots per treatment. Trials were repeated at least once but because the overall trends were similar, the results of only one trial are presented.

Dos response curves for Zohar LQ-215 and fenarimol

Dose response curves for Zohar LQ-215 and fenarimol were used to compare the efficacy of the two compounds. Trials were conducted using a modified leaf-disks test [Cohen, 1993]. Leaf disks (9 mm in diameter) were removed from leaves by means of a cork borer. Sampling was done after the leaves had been treated with the compounds. The elapsed time between spraying and inoculation was 2 h. Leaf disks were placed in Petri dishes containing 0.16% water agar amended with 25 mg ml^{-1} benzimidazole (Sigma B 9131) which in the concentration used, is non-toxic to the pathogen and prevented early senescence of the leaf disks. Leaf disks were inoculated using one 10-ml drop of water containing 300–500 conidia of *S. fuliginea*. The drops were left to dry. Petri dishes were then placed in a growth chamber (Conviron G-30, Winnipeg, Canada) with a 12-h photoperiod and low light intensity of 30 μE m^{-2} sec^{-1}, at 25 °C to enhance the development of *S. fuliginea*. The development of powdery mildew hyphae and conidia on the leaf disks was assessed 7 days after inoculation using a binocular stereoscope (magnification × 100) and the frequency of infected disks was determined. Leaf disks were considered infected if mycelium and/or sporulation were observed. Ten leaf disks were placed in each Petri dish and there were three replicate dishes for each concentration of the tested compounds.

Effects of LQ-215 on S. fuliginea

The effects of Zohar LQ-215 and fenarimol on spore germination and germ-tube elongation were evaluated by means of the leaf disk test described above.

Cucumber leaves were soaked in aqueous solutions of the compounds (Zohar LQ-215 at concentrations of 0.1, 0.2, 0.4 and 0.8%; fenarimol at concentrations of 0.01, 0.02, 0.05 and 0.1%) for 30 sec. The leaves were left to dry at room temperature for 3 h; leaf disks were then cut, placed in Petri dishes on benzimidazole-amended water agar and inoculated with *S. fuliginea* by blowing air from an infected cucumber plant towards the leaf disks. Petri dishes were then placed in a growth chamber with a 12-h photoperiod under a light intensity of 30 μE m^{-2} sec^{-1}, and 25 °C. After 24 h the leaf disks were bleached by soaking in a solution of 2% HCl for 3 h to facilitate observation of the germinating spores. The leaf disks were then stained by immersion for 1 min. in boiling solution of trypan blue (0.02%, Sigma 0887) dissolved in lactophenol [Elad *et al.*, 1989]. Spore germination was determined by means of a microscope (magnification × 250). Four samples (replicates) of 20–25 spores were counted for each concentration of Zohar LQ-215 and fenarimol.

Possible effects of Zohar LQ-215 on infection or on external mycelium development and sporulation of *S. fuliginea* were evaluated in the greenhouse. Cucumber plants were grown and inoculated as described above. The detergent was applied at a concentration of 0.4%, 2 h or 5 days after inoculation. The earlier application was conducted to evaluate possible effects on infection and establishment, whereas the later application was conducted to evaluate possible effects on external mycelial growth and sporulation. Control plants were inoculated but not treated with Zohar LQ-215. After the appearance of symptoms (on day 7) disease development on inoculated leaves was evaluated at 5–7 days intervals.

The duration of the efficacy of Zohar LQ-215 in *S. fuliginea* suppression relative to that of the fungicide fenarimol was evaluated in a greenhouse trial. The detergent was applied once (at a concentration of 0.2%), 5 days after inoculation. Control plants were inoculated but not treated. After the appearance of symptoms, disease development on inoculated leaves was evaluated at 5–7 day intervals.

The persistence of Zohar LQ-215 and fenarimol was examined in a trial conducted in a controlled environment. Three leaves of cucumber plants were sprayed to run-off with 0.4% of Zohar LQ-215 solution or 0.1% fenarimol solution. The plants were then placed in a growth chamber with a light intensity of 300 μE m^{-2} sec^{-1} for 3 h, 2 days or 4 days. Leaves were then sampled and leaf disks were cut, inoculated and incubated as described above. The development of powdery mildew hyphae and conidia on the leaf disks was observed and recorded 7 days after inoculation using a binocular stereoscopic microscope (magnification × 100) and the frequency of infected leaf disks was determined. There were four replicates (Petri dishes each containing 10 leaf disks) for each exposure time (i.e., 3 h, 2 and 4 days) of the tested compounds.

Interaction between Zohar LQ-215 and fenarimol

Possible interaction between the effects of Zohar LQ-215 and the fungicide fenarimol was evaluated in greenhouse and field trials. The following treatments were included in both trials: 1) untreated control; 2) application of Zohar LQ-215 alone at full rate; 3) application of fenarimol alone at full rate; 4) application of fenarimol at half rate and 5) application of a mixture of Zohar LQ-215 at full rate and fenarimol at half rate. In the greenhouse trial, Zohar LQ-215 was applied at a concentration of 0.2% and fenarimol at rates of 0.1% (full rate) and 0.05% (half rate). Sprays were applied once, 5 days after inoculation.

In the field, seedlings were sown (two per hole) on 3 May, 1993 in the Newe Ya'ar Research Center in northern Israel. Plants were spaced at 50 cm within rows and 180 cm between rows. The crop was drip irrigated and fertilized according to the common cultural practices in that region in the spring. Herbicides and insecticides were applied as needed. The experiment was laid out in a randomized complete block design with four replicates per treatment. Each experimental plot was 1.8 × 7 m, containing 28–30 plants. Sprays were applied by means of a motorized knapsack sprayer equipped with a blower. Each experimental plot was sprayed with a volume of approximately 1 L. Zohar LQ-215 was applied at a concentration of 0.3% and fenarimol at 0.1% (full rate) and 0.05% (half rate). Zohar LQ-215 (treatment 2) was applied at weekly intervals, for a total of six sprays (8^{th}, 14^{th}, 21^{st} and 28^{th} June, and 5^{th} and 7^{th} July). Fenarimol and its mixture with Zohar LQ-215 (treatments 3, 4, and 5) were applied at biweekly intervals, for a total of three sprays (8^{th}, 21^{st} June and 5^{th} July). Spraying was initiated at flowering, before powdery mildew symptoms were observed in the field. Disease severity was assessed visually at 5–7 day intervals. Twenty fully expended leaves were arbitrarily selected from each experimental plot and disease severity was evaluated as described above. Data (from all trials) were subjected to statistical analysis; whenever the F values were significant at $P < 0.05$, treatments were compared according to Fisher's protected LSD test.

The joint action of Zohar LQ-215 and fenarimol in powdery mildew suppression was evaluated by means of the Abbott formula [Levi *et al.*, 1986]. It was assumed that the joint actions of the compounds in the mixture were independent (i.e., the presence of one compound did not affect the efficacy of the other). Thus, the expected efficacy of the mixture is:

$$E_{(exp)} = a + b - (ab/100)$$

in which $E_{(exp)}$ is the expected control efficacy of the mixture, and a and b represent the proportion of disease control achieved by Zohar LQ-215 and fenarimol, respectively. The value of ab represents the proportion of disease control achieved by both compounds together. For synergy calculations, the ratio (SF, synergy factor) between the observed experimental efficacy of the mixture $E_{(obs)}$ and the expected efficacy $E_{(exp)}$ in the mixture was computed:

$$SF = E_{(obs)}/E_{(exp)}$$

A ratio of SF greater or smaller than 1 indicates a deviation from the hypothesis of independent action, and indicates a biological interaction between the compounds. If $SF > 1$, there is synergism; if $SF < 1$, there is antagonism.

Results

Dose response curves for Zohar LQ-215 and fenarimol

The efficacy of Zohar LQ-215 in *S. fuliginea* suppression was compared with that of the fungicide fenarimol. As expected, control efficacy increased with increasing concentration of the compounds. The ED_{50} values were calculated from the dose-response curves and were 0.012% for fenarimol and 0.356% for Zohar LQ-215. In addition, differences between the two compounds in the slope of the dose response curve were not significant (Fig. 1).

Effects of Zohar LQ-215 on S. fuliginea

Zohar LQ-215 did not significantly affect spore germination or germ-tube elongation at the concentrations tested. The frequency of spore germination on leaves treated with Zohar LQ-215 at concentrations of 0.1–0.8% was 82.1–92% of the germination on untreated leaves. The length of the germ-tubes on treated leaves was 90–100 μm, compared with of 80–120 μm in the controls. Fenarimol, on the other hand, inhibited both processes significantly. Spore germination and germ-tube elongation decreased from 39% and 88 μm on leaves treated with 0.01% fenarimol to 6% and 43 μm on leaves treated with the fungicide in a concentration of 0.1%. Application of the detergent at a concentration of 0.4% on the day of inoculation had no effect on powdery mildew development, and disease severity in treated plants did not differ significantly from that in untreated plants. However, when a similar concentration was applied 5 days after inoculation disease severity was significantly reduced (Fig. 2).

The persistence of Zohar LQ-215 on leaves was relatively short compared to that of fenarimol. Four days after application, Zohar LQ-215 did not inhibit powdery mildew development, indicating that by that time the product had been degraded to an ineffective concentration. Fenarimol, on the other hand, was still effective 4 days after application (Fig. 3). In the greenhouse experiment carried out during development of

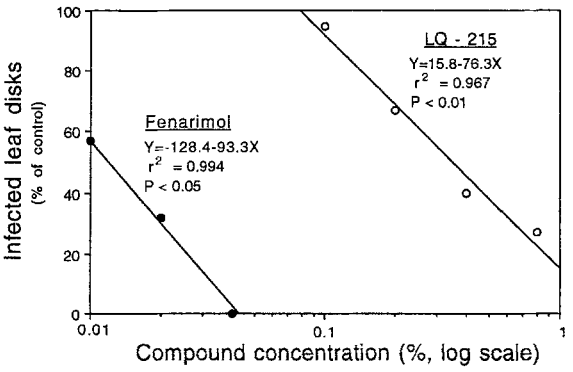

Fig. 1. Dose-response curve of the effect of the detergent Zohar LQ-215 and fenarimol on *Sphaerotheca fuliginea*. Responses were determined by means of a leaf disk test.

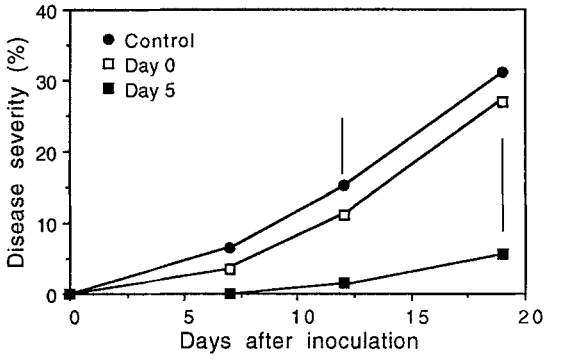

Fig. 2. Effects of timing of a single application of the detergent Zohar LQ-215 (applied at 0.4%) on *Sphaerotheca fuliginea* development in cucumber plants. Bars indicate the LSD values.

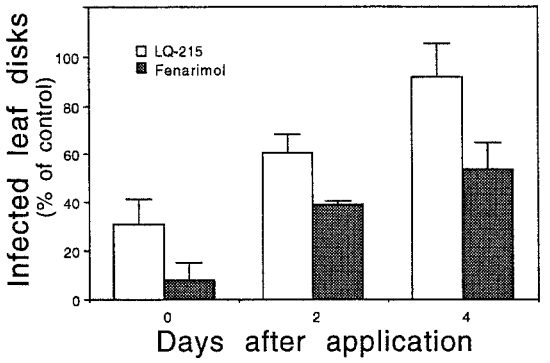

Fig. 3. Degradation of Zohar LQ-215 and fenarimol, as assessed by a leaf-disk test. The frequency of infected leaf disks was recorded 7 days after inoculation which was 0,2 or 4 days after treatment. Bars indicate the SE.

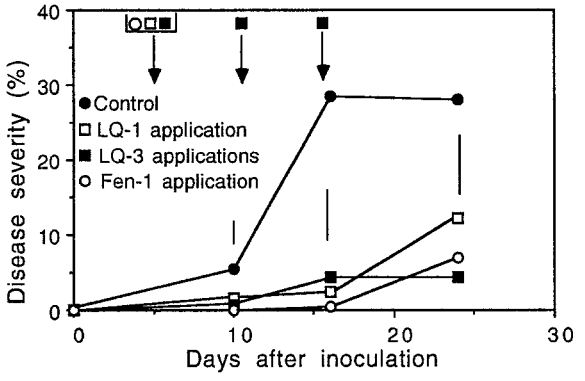

Fig. 4. Effects of Zohar LQ-215 and fenarimol on the development of *Sphaerotheca fuliginea* in greenhouse-grown cucumber plants. Arrows indicate the time of spraying. Bars indicate the LSD values.

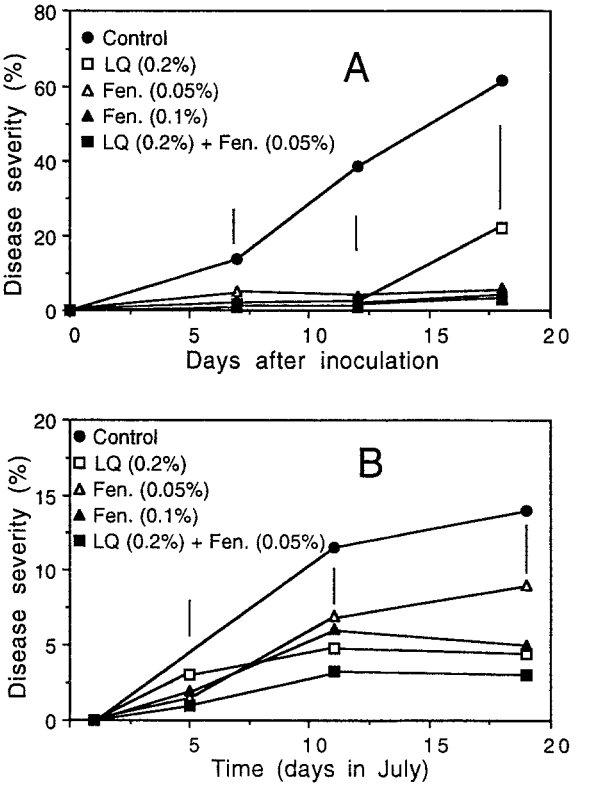

Fig. 5. Effects of Zohar LQ-215 and fenarimol, applied separately and mixed, on *Sphaerotheca fuliginea* on cucumber grown in the greenhouse (A) or in the field (B). Bars indicate the LSD values.

a polycyclic epidemic, the effects of Zohar LQ-215 were apparent for 24 days after spraying. When it was applied three times (at 5-day intervals), the detergent maintained a relatively low disease severity (2.4%) for the duration of the experiment (Fig. 4).

Interaction between Zohar LQ-215 and fenarimol

In the greenhouse, all treatments significantly suppressed disease relative to that occurring on untreated plants and differences among the treatments were insignificant (Fig. 5A). Disease severity on plants treated with fenarimol at full rate or at half rate was very low (6%, compared with 62% on untreated plants). Thus, a mixture of Zohar LQ-215 with fenarimol at half rate did not improve disease suppression more then did fenarimol at half rate, alone (Fig. 5A).

In the field, all spraying treatments decreased disease severity significantly relative to that of untreated plots. However, by the end of the growing season the efficacy of fenarimol applied three times at half rate was significantly less than that of Zohar LQ-215 (six applications) or fenarimol applied at full rate (3 applications). A mixture of fenarimol at half rate and Zohar LQ-215 (three applications) was effective, and disease reduction resembled that achieved by applying fenarimol at full rate (Fig. 5B). The joint action of Zohar LQ-215 and fenarimol in powdery mildew suppression was determined by means of the Abbott formula. The observed control efficacy of the Zohar LQ-215 and fenarimol mixture was 78.5% whereas the predicted efficacy was 79.3%. The value of the *SF* (0.99) was close to 1, indicating that the effects of the two compounds in the mixture were additive.

Discussion

The results of trials conducted in the present study confirmed previous observations concerning the efficacy

of the detergent Zohar LQ-215 against *S. fuliginea*. Compared with fenarimol, a fungicide commonly used for the suppression of powdery mildew, Zohar LQ-215 had a relatively low efficacy: ED_{50} for the detergent was 30-times as great as that for the chemical fungicide (Fig. 1). However, low toxicity makes Zohar LQ-215 an attractive candidate for integrated pest management (IPM) programmes. Another important outcome of the dose-response trial is that the regression slopes did not differ significantly between the detergent and the chemical fungicide (Fig. 1). Similar slopes indicate that the response of the pathogen population varied similarly to both compounds. This result is important because it has implications for application of the compounds in the field. However, conclusions derived from this test may be considered only as an indication. In order to attain more general and reliable conclusions the response of many more isolates of *S. fuliginea* should be tested.

The detergent Zohar LQ-215 did not affect spore germination and germ-tube elongation of *S. fuliginea* at the concentrations tested (up to 0.8%). When applied 2 h after inoculation, the detergent did not reduce disease development significantly, relative to disease development in untreated plants. However, when it was applied prior to symptom appearance (5 days after inoculation), disease severity was significantly reduced (Fig. 2). Mycelium of *S. fuliginea* develops externally, and only the haustoria penetrate into the host cells [Sitterly, 1978]. Moreover, the fungal structures contributing most to visual disease assessment are conidia and conidiophores. Thus, the above results may indicate that the first stages of powdery mildew development (i.e., spore germination and germ-tube elongation) are less sensitive to the detergent than the later stages of the disease cycle (conidiophore and conidial production). It might therefore be concluded that Zohar LQ-215 inhibited sporulation.

An important feature of any compound applied against plant pathogens is its persistence. Although Zohar LQ-215 in its present formulation degraded relatively quickly (Fig. 3), it had a relatively long effect on the development of the *S. fuliginea* epidemic; when applied at a rate of 0.2%, Zohar LQ-215 suppressed powdery mildew significantly for about 24 days (Figs. 2 and 4). A similar effect was achieved by applying the fungicide fenarimol (Fig. 4). In the field, Zohar LQ-215 applied on a 7-day schedule significantly suppressed powdery mildew for the duration of the experiment (Fig. 5B).

Zohar LQ-215 can be effectively integrated in an IPM program. In our study the possibility of reducing the quantity of chemical fungicide by mixing a reduced rate of the chemical with the detergent was tested. Disease suppression achieved by the mixture was as effective as that by the fungicide applied at full rate (Fig. 5). Analysis of the results revealed that in the field trial the effects of the detergent and the fungicide were additive. Another possibility for integration of Zohar LQ-215 into a control program is to apply it in alternation with a fungicide. this possibility was not examined in our study. Although the conclusions derived from the present work are encouraging, the integration of Zohar LQ-215 and a fungicide should be further examined under field conditions before being recommended to commercial growers.

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