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Use of Quantitative Models to Describe the Efficacy of Inundative Biological Control of Fusarium Wilt of Cucumber

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Fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* is a serious vascular disease worldwide. Biological control of Fusarium wilt in several crops has been accomplished by introducing non-pathogenic *Fusarium* spp. and other biocontrol agents in soil or in infection courts. In this study, quantitative models were used to determine the biocontrol efficacy of inundatively applied antagonist formulations and the length of their effectiveness in controlling Fusarium wilt of cucumber. Quantitative model of the form [$Y=L\ (1-\exp^{-kX})$] best described the relationship between disease incidence (Y, %) and inoculum density (X) of isolates F51 and F55. Isolate F51 was selected as a more virulent isolate based on the extent of its effectiveness in causing the wilt disease. The degree of disease control (X_i/X) obtained with the density of the biocontrol agent (Z), was described by the model [$X_i/X=A\ (1-\exp^{-CZ})$]. The zeolite-based antagonist formulation amended with chitosan (ZAC) was better at lower rates of application and peaked at around 5 g/kg of the potting medium, whereas the peat-based antagonist formulation (PA) peaked at around 10 g/kg of the potting medium. ZAC formulation provided significantly better suppression of Fusarium wilt as described by the curvilinear relationship of the type $Y=a+bX+cX^2$, where Y represents percent disease incidence and X represents sustaining effect of the biocontrol agent.

Keywords **:** Biocontrol, *Fusarium oxysporum*, quantitative models

Cucumber (*Cucumis sativus* L.) is a crop of high economic importance in many countries. Meanwhile, Fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum*, is a serious vascular disease worldwide (Vakalounakis, 1993). Biological control of Fusarium wilt of several crops has been accomplished by introducing non-pathogenic *Fusarium* spp. in soil or in infection courts. Alabouvette et al. (1996) reported that suppression was due to nutrient competition between pathogenic and saprophytic *Fusarium* spp., whereas Schneider (1984) suggested that reduced incidence of Fusarium wilt of celery induced by non-pathogenic *Fusarium* spp. was directly correlated with competition for infection sites at the root surface. Fluorescent pseudomonads have also been applied to seeds and rhizosphere soils or other infection courts for biological control (Alabouvette et al., 1996; Weller, 1988).

Previous studies (Singh et al., 1999) reported the efficacy of two chitinolytic bacterial strains, *Paenibacillus* sp. (# 300) and *Streptomyces* sp. (# 385), in suppressing Fusarium wilt of cucumber in soil-less potting medium by efficiently producing the hydrolytic enzymes, chitinase and β-1,3 glucanase. The focus of most of the studies dealing with biological control of the disease has been either on testing the efficacy of different preparations or understanding the mechanism of action of biological control agents. While such studies are important for identifying the effective biological control agents, yet these have little extrapolatibility and thus, the results can not be generalized. Also, sometimes it is difficult to prescribe an optimum dose vis-à-vis disease control that may fall within a set of concentrations of the biological agent being studied. Johnson (1994) proposed the use of a quantitative model, the parameters of which could be used to predict efficacy and expected behavior of inundatively applied biological control agents. This paper presents the use of such quantitative models (Johnson, 1994) to determine the biocontrol efficacy of inundatively applied antagonist formulations and the length of their effectiveness in controlling Fusarium wilt of cucumber.

Materials and Methods

Isolation of pathogen and pathogenicity tests. *F. oxysporum* f. sp. *cucumerinum* was isolated from cucumber plants showing

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typical wilt symptoms on Komada agar (Komada, 1975) and sub-cultured on potato dextrose agar (PDA, Difco). To prepare inoculum of the pathogen, a chopped potato soil mixture consisting of 50 g of potato (cut into small pieces) and 500 g of field soil (app. 25% wt/wt moisture) were placed in 2-liter Erlenmeyer flasks autoclaved for 1 hour, and then inoculated with PDA plugs (0.5 cm in diameter) of *F. oxysporum* f. sp. *cucumerinum*. After 4 weeks of incubation at 30°C, the inoculum was air dried and screened to yield pieces of inoculum 1-2 mm in size. The pathogenicity tests of two isolates F51 and F55 were done on cucumber seedlings of variety 'F_1 Summer long' (Seoul Seed Co., Korea) at two- to three-true leaf stage. The inoculum of each isolate at 0.5, 1.0, 2.5, and 5.0 g of inoculum was added per kg of a commercial soil-less peat based potting medium (Punong Co., Kyoungju, Korea). Each rate of inoculum was mixed thoroughly to ensure uniform distribution and then placed in 10-cm plastic pots with perforated bases.

Preparation of antagonist formulations and studies on inundative biological control. Antagonistic bacteria were grown at 30°C in 2-liter Erlenmeyer flasks containing 500 ml of sterilized Tryptic Soy Broth (TSB) on a rotary shaker for 96 hours at 150 rpm. The cells were harvested by centrifugation at 5000 g for 15 minutes, and the pellets were re-suspended in 10 ml of sterile distilled water. Cell suspensions of *Paenibacillus* sp. (# 300) and *Streptomyces* sp. (# 385) (referred as A) (av. 3×10^{11} cfu/ml) were mixed in 1:1 combination (vol /vol) before these were added into the pathogen-infested potting medium. Fifteen (15) milliliters of the suspension was added to 1 kg of the potting medium in polythene bags and the medium was stirred vigorously for even distribution of the bacteria. To prepare antagonist formulations, a 10-ml aliquot of a suspension of two antagonists mixed in 1:1 combination was added to sterilized Canadian peat moss (4 g), previously adjusted to pH 7.0 with $CaCO_3$ and mixed thoroughly (referred as PA). In ZAC formulation, the peat was replaced by Zeolite (Wangpyo Chemical Co., Ltd., Pohang, Korea) to which 10-ml cell suspension of antagonists supplemented with 0.25% (wt/vol) chitosan was added.

Three (3) grams of inoculum of *F. oxysporum* f. sp. *cucumerinum*, isolate F51 was added per kg of potting medium into the polythene bags. Preparations of antagonists either A, PA, or ZAC were incorporated into the pathogen-infested potting medium, remixed, and then added to 10-cm plastic pots, perforated at the base, each containing 180 g of the mix.

Pathogenicity tests, as well as biological control assay studies, were carried out in a plant growth chamber at 28°C/24°C (day/night) and 75% relative humidity for 30 days. The plants received a 12/12 hour-light/darkness cycle (cool white fluorescent light). Five cucumber seeds were planted in each pot. Each treatment was replicated five times with each pot serving as a replicate.

Application of quantitative models to study biocontrol efficacy. The quantitative model (Eqn. 1) proposed by Johnson (1994) was fitted to the disease incidence vs. inoculum density data. The parameters of the model were estimated by non-linear least square method using Marquardt algorithm.

$$Y=L\ (1-\exp^{-kX}) \quad (1)$$

Where L, an asymptote, represents proportion of the host tissue available for infection, X is the inoculum density of the pathogen, k is a constant that governs the effectiveness of the inoculum, and Y is the proportion of the disease. An asymptote, 'L' in the model indicates that 100% of the host tissue is not susceptible to infection. Therefore, a disease level of 100% is never attained.

The effect of dose of biocontrol agent (X) on disease control (X_i/X) was best described by the following model (equation 2).

$$X_i/X=A\ (1-\exp^{-CZ}) \quad (2)$$

Where Z is the density of the biocontrol agent, C is a constant that governs the efficacy of the biocontrol agent, A is an asymptote, and X_i/X is the proportion of the inoculum units of the pathogen population rendered ineffective by the biocontrol agent. The equation (2) is based on the assumption that the mechanism of action of the biocontrol agent is independent of its own density and X_i/X remains constant over a range of pathogen densities (Johnson, 1994).

Results and Discussion

The influence of inoculum density of two isolates of *F. oxysporum* f. sp. *cucumerinum*, F51 and F55, on disease incidence due to wilt is shown in Fig. 1. The following quantitative models (equations 3 and 4) best described the relationship between inoculum density (X) and disease incidence (Y) for isolates F51 and F55, respectively.

$$Y=79.8\ (1-e^{-1.392\ X}) \quad (3)$$

$$Y=71.7\ (1-e^{-3.747\ X}) \quad (4)$$

It is apparent from the equations that the two isolates differed considerably with respect to the extent of disease

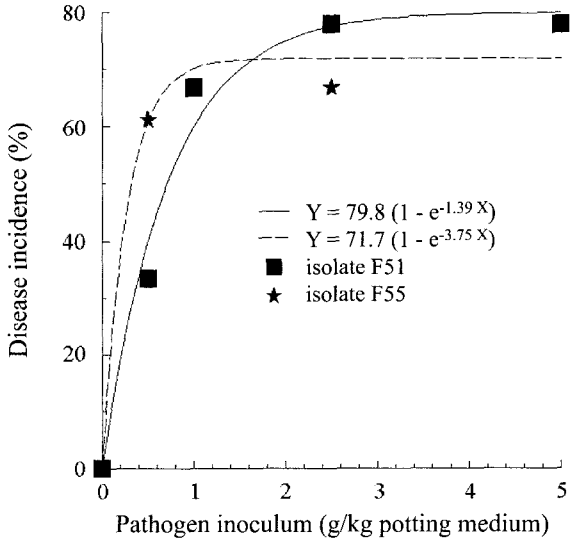

Fig. 1. Percent disease incidence in response to different inoculum doses of *Fusarium oxysporum* f. sp. *cucumerinum* isolates, F51 and F55.

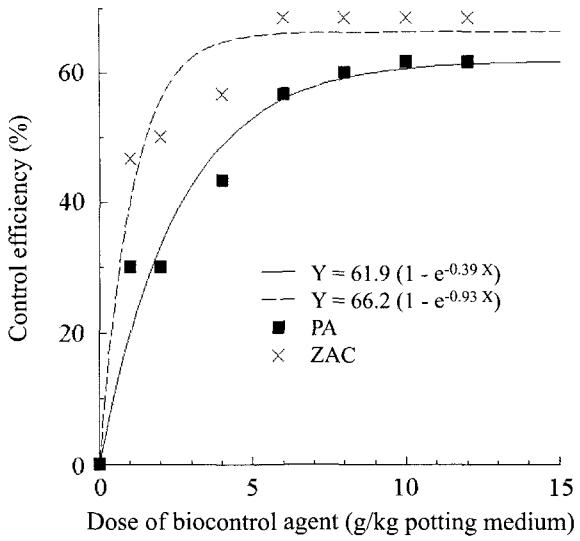

Fig. 2. Effect of dose (g/kg potting medium) of biocontrol agents on the control of wilt pathogen *Fusarium oxysporum* f. sp. *cucumerinum*. PA represents peat-based formulation of antagonists *Paenibacillus* sp. (# 300) and *Streptomyces* sp. (# 385). ZAC represents zeolite-based antagonist formulation prepared by mixing zeolite (4 g) with 10 ml of mixed cell suspension of the two antagonists amended with 0.25% (wt/vol) chitosan.

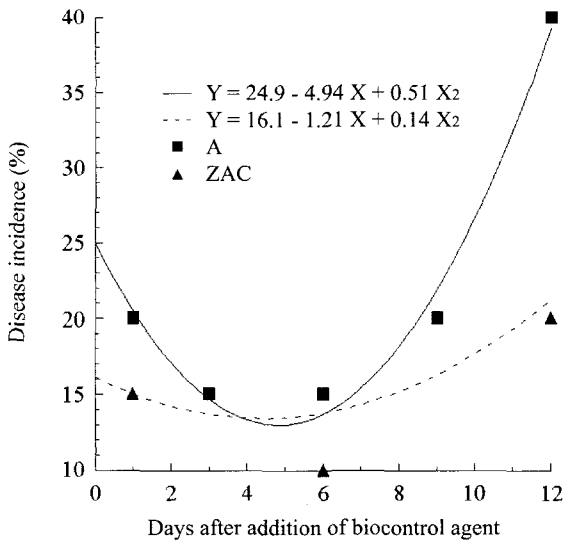

Fig. 3. Sustaining effect of dose (g/kg of potting medium) of biocontrol agents on the suppression of Fusarium wilt of cucumber. A represents antagonist mixed cell suspension (1:1 vol/vol) of *Paenibacillus* sp (# 300) and *Streptomyces* sp (# 385). ZAC represents zeolite-based antagonist formulation prepared by mixing zeolite (4 g) with 10 ml of mixed cell suspension of the two antagonists amended with 0.25% (wt/vol) chitosan.

incidence and their effectiveness in causing the wilt. The value of asymptote shows that isolate F51 was more penetrative, as about 80% of the host tissue was infected as compared with 71.7% due to isolate F55. However, at inoculum density <1 g/kg of the potting medium, isolate F55 caused greater infection and attained a plateau around that value. In contrast, the effect of F51 continued to increase to an inoculum density of about 2-4 g/kg of the potting medium.

The influence of biocontrol agent dose (both PA and ZAC formulations) on the suppression of wilt incidence (Y) is shown in Fig. 2. Both PA and ZAC formulations were effective in controlling the pathogen. However, ZAC was relatively more effective as it could control a maximum of 66% plants to remain "wilt" free (equation 5) as compared with 62% by PA formulation (equation 6).

$$Y=66.241\ (1-e^{-0.934X}) \quad (5)$$

$$Y=61.88\ (1-e^{-0.3898X}) \quad (6)$$

The effect of ZAC formulation was higher at low rates of its application and it tended to attain a plateau around 5 g/kg of the potting medium. The effect of PA formulation was slower but steady and it attained a plateau at around 10 g/kg of the potting medium. This indicates that in order to attain similar levels of pathogen control, relatively higher dose of PA is required than ZAC formulation.

Antagonists formulations amended with chitosan have been shown to increase the efficacy of the biological control of *R. solani* (Chung, 1997; Sung and Chung, 1997). In addition to the role of chitosan as the substrate for antagonists, its function in inducing host resistance when added directly to the soil has been reported in cucumber plants (Ghaouth et al., 1994; Singh et al., 1999). The sustaining effect of biocontrol agent on disease incidence for two typical cases is shown in Fig. 3. The antagonist formulation had a significant effect on the suppression of Fusarium wilt. The ZAC formulation provided significantly better suppression of Fusarium wilt as compared with the non-treated control and the formulations PA and ZA. Treatment with the PAC formulation also resulted in significantly lower wilt incidence than the control. Curvilinear relation of the type $Y=a+bX+cX^2$ best described the data, where Y represents percent disease incidence and X represents sustaining effect of the biocontrol agent, i.e. the efficacy days after biocontrol agent addition. The regression coefficients (a, b and c) and coefficients of determination (R^2) are presented in Table 1. The coefficient of determination for different formulations varied from 0.55 with ZAC to 0.99 with A. The intercept values (a) show that the extent of disease incidence was highest in ZA, followed by A, PAC, PA and least in ZAC. Plots of the data for two typical cases (A and ZAC) are shown in Fig. 3. The line for A shows that the efficacy of biocontrol agents used as such in 1:1 combination is maximum at 3-6 days, which tends to decline as the time progresses. The disease incidence was

Table 1. Regression coefficients (a, b, and c) and coefficient of determination (R^2) for the curvilinear relationship ($Y=a+bX+cX^2$) between disease incidence (Y, %) and the sustaining effect of biocontrol agent (X)

Treatment	a	b	c	R^2
A	24.89	4.935	0.511	0.99
PA	19.22	1.984	0.241	0.74
ZA	30.36	6.467	0.604	0.99
ZAC	16.10	1.207	0.136	0.55
PAC	22.54	4.278	0.406	0.873
Control	90.85	6.671	0.452	0.95

around 45% at day 12-15. Whereas, in ZAC formulation, the efficacy of biocontrol agent was relatively stable, as indicated by b coefficient. Even after 15 days of application, the biocontrol agent was still able to contain the disease which was just around 20%.

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