An antifungal protein of the tropical forage legume *Clitoria ternatea* controls diseases under field and greenhouse conditions

S. KELEMU, G. Mahuku and G. Segura

Crop and Agroecosystem Health Management
Centro Internacional de Agricultura Tropical (CIAT), A. A. 6713, Colombia, South America

ABSTRACT

Plants defend themselves from pathogens and insect pests by a variety of ways, including the production of proteins with antimicrobial and/or insecticidal properties. We previously reported the isolation, purification and characterization of a highly basic small protein from seeds of *Clitoria ternatea*. This protein, designated 'finotin', has broad and potent antifungal, antibacterial and insecticidal properties, raising the possibility that finotin may contribute to the high level of disease and insect resistance observed in *C. ternatea* in the field. In this study, we examined the effect of direct applications of crude protein preparations on diseases of tomato, beans and the tropical forage *Brachiaria* under field and greenhouse conditions. The protein has potent *in vitro* growth inhibitory effect on the bean angular leaf spot pathogen *Phaeoisariopsis griseola*, the rhizoctonia foliar blight disease pathogen *Rhizoctonia solani* on species of *Brachiaria*, and various important plant pathogenic fungi. Plants sprayed with the crude protein preparation consistently developed fewer disease lesions of bean angular leaf spot, rhizoctonia foliar blight, and diseases of tomato, than the control plants both in greenhouse and field experiments. The potential use of finotin as a biopesticide for disease control will be discussed.

INTRODUCTION

Seeds use strategies such as production of antimicrobial and/or insecticidal proteins to germinate and survive in soils that are densely inhabited by a wide range of microfauna and microflora. Antimicrobial proteins and peptides have been isolated from seeds of maize (*Zea mays* L.), radish (*Raphanus sativus* L.) and various other plants. They are believed to play a role in plant defense because of their strong antimicrobial activity. This belief is supported by their ability to confer resistance (to pathogens) to transgenic plants containing genes that encode them.

In a previous study, we examined seeds from several tropical forage legumes, for antifungal properties. Of those examined, we isolated, purified, and characterized a protein, designated 'finotin', from seeds of *Clitoria ternatea* (L.) that exhibited, *in vitro,* strong antifungal activity on the test fungus *Rhizoctonia solani* Kühn (Kelemu et al., 2004). This protein has antifungal, antibacterial and insecticidal properties.
In this study, we examined the potential use of finotin as a biopesticide for disease control under field and greenhouse conditions.

MATERIALS AND METHODS

Treatment of P. griseola conidia with the protein finotin: Twenty-µl of a conidial suspension (10^{-4}) was placed on a slide and subsequently covered with a thin layer of potato dextrose agar (PDA) medium. A 200-µl crude antifungal protein preparation (the same concentration that was used to spray onto bean plants) was applied on the agar. Protein preparation protocols were as described previously (Kelemu et al. 2004). Control slides had PDA and water. These were placed in Petri dishes containing wet filter paper and incubated at room temperature. Pictures of conidia were taken under the microscope at 0, 32 and 96 hours to observe the development of individual conidia.

Plant inoculation and extract applications: A highly virulent isolate of the pathogen *P. griseola* was grown on V8 agar at 24°C for 12 days. Conidia were collected and suspended in sterile distilled water at a concentration of 2×10^4 conidia per mL. This inoculum was used on *Phaseolus vulgaris* variety Sprite (a susceptible one) bean plants.

Greenhouse testing: Seventeen-day old bean plants (15 plants per treatment) were sprayed with, either the fungicide benlate (500 µg/ml), crude antifungal protein preparation, or sterile water. Two hours later all the plants were inoculated with *P. griseola* conidia. The inoculated plants were placed in a humidity chamber for 4 days, then transferred to the greenhouse for symptom development. Treatments with crude antifungal protein, benlate or sterile water continued every 2 days. Disease evaluations were conducted 10 days after inoculation.

Field testing: Thirty days old seedlings of tomato variety Manalucie were transplanted to the field in a randomized design with 3 replications (8 plants per treatment in each replication). Treatments were; 1) control treatment with water alone, 2) spray application (till plants were completely wet) of crude protein preparation once a week, and 3) spray application of crude protein twice a week. Various diseases developed under natural infections.

RESULTS AND DISCUSSION

Effect of antifungal protein Finotin on bean angular leaf spot: The crude protein extract from seeds of *C. ternatea* CIAT 20692 showed antifungal activity *in vitro* on the pathogen *P. griseola* (data not shown). Conidia treated with the crude protein failed to germinate 32 or 96 hours after treatment (Figure 1). Plants treated with the crude antifungal protein preparation consistently developed fewer angular leaf spot disease lesions than the control plants that were treated with sterile distilled water [Figures 2, 3]. Had a purified protein been used to control the disease on bean plants, the level of disease control would perhaps have been even higher.

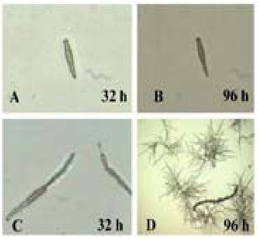

Figure 1. Treatment of *Phaeoisariopsis griseola* conidia with the antifungal protein finotin. Conidia failed to germinate in the presence of the antifungal protein finotin, 32 and 96 hours (A and B) after treatment, whereas those treated with sterile water germinated (C and D).

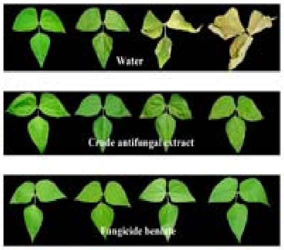

Figure 2. Treatment of bean plants with crude protein extract from seeds of *C. ternatea* CIAT 20692 against the fungal pathogen *P. griseola*, causal agent of angular leaf spot disease. The fungicide benlate was used as a positive control.

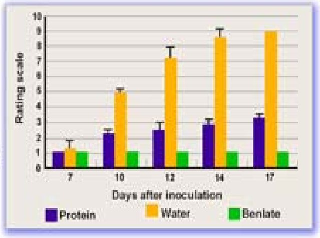

Figure 3. Angular leaf spot disease development in artificially inoculated bean plants following treatment with crude antifungal protein preparations isolated from C. ternatea CIAT 20692, the fungicide benlate, or water control

Effect of antifungal protein finotin on tomato diseases: Tomatoes are generally susceptible to a number of diseases under natural conditions. The purpose of these experiments is to develop a simple disease control strategy for small producers using this antifungal protein. Plants sprayed with thecrude protein preparation once or twice a week developed better, had fewer disease symptoms, had more plant biomass, and produced more tomatoes than control plants (Figures 4, 5

Figure 4. Tomato plants sprayed with crude antifungal protein preparations twice (3), or once (2) a week, and control (water) [1].

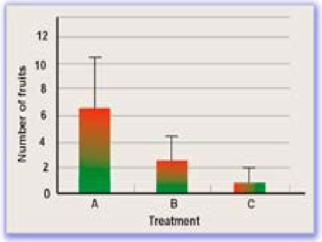

Figure 5. Average tomato fruit yield per tomato plant in plants treated with crude protein preparations twice a week (A), once a week (B) and water only (C).

The protein finotin, is shown to be inhibitory to the growth of a range of important plant pathogenic fungi and at least one important bacterium pathogenic to common bean, as well as two important species of bruchids, *Z. subfasciatus* and *A. obtectus* (Kelemu et al., 2004) These findings raise the possibility that finotin may contribute to the high level of disease and insect resistance observed in *C. ternatea* in the field. Finotin is released from seeds when the seed coat is mechanically damaged creating a zone of fungal growth inhibition *in vitro*. The antifungal activity of finotin is not affected by high temperatures, which made attractive for the direct use of this protein in disease management under field and greenhouse conditions. The results presented here demonstrate that a disease control strategy can be developed for small producers using this antifungal protein.

REFERENCE

Kelemu, S., Cardona, C. and Segura, G. 2004b. Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea* (L.), a tropical forage legume. Plant Physiology and Biochemistry 42 (11): 867-873.