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Field Management of Late Blight of Tomatoes Caused by *Phytophthora infestans* Using Antibiotics from *Streptomyces* species

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Abstract Antibiotic culture filtrates from *Streptomyces* isolates coded 28P and CS35 were tested for efficacy in the control of late blight of tomatoes under field conditions. Crude culture filtrates, cell free culture filtrates, concentrated culture filtrates and a mixture of the two cell free culture filtrates were evaluated. Dithane M45 was used as a standard chemical check. The experiment was laid out in a Randomized Complete Block Design (RCBD) replicated four times with each treatment appearing once in each block. Concentrating culture filtrates by removing 30% of water enhanced their efficacy and the various culture filtrates had a significant ($p<0.05$) effect in controlling late blight disease. Mixing culture filtrates did not have significant ($p<0.05$) effect in improving efficacy in late blight management. Concentrating culture filtrates beyond 50% resulted in phytotoxic effects. The isolates had antifungal activity against *Phytophthora infestans* and with enhancement of this activity they can be used to manage the disease either alone or in IPM programmes.

Key words: Antibiotics, Culture filtrates, Isolates, *Streptomyces*, Tomato

Introduction

The tomato crop is an important horticultural crop in Kenya. It is produced and consumed by majority of households. Its production is hampered by poor soil fertility, unreliable rainfall patterns, poor marketing structures, post-harvest handling problems and most important, pests and diseases (HCDA, 1996). Late blight ranks as the most important disease problem of the tomato. It is favored by cool humid conditions and its spread is very fast under favorable conditions. *Phytophthora infestans* sporulates most abundantly at relative humidity near 100% and at temperatures between 16 and 22^{o}C (Hartman & Huang, 1995). It causes up to 100% crop losses if not controlled (HCDA, 1996). Late blight is a very damaging disease of potatoes and tomatoes and it is a threat to food security since many resource poor farmers cannot afford the numerous fungicides required to control the disease (Chycoski, 1996. The disease can cause total destruction of all plants within a week or two when weather conditions are favourable (Agrios, 1998). Late blight is found in nearly all areas where potatoes and tomatoes are grown but is more severe in humid high rainfall areas (Hartman & Huang, 1995). Farmers try to control the disease through various ways but each of these control measures has considerable limitations. Fungicides are used to control the disease but numerous fungicide applications (7-8 sprays) are needed (Legard & Fry, 1995), the pathogen develops resistance (Lashomb & Casagrande, 1981) and chemical residues in the harvested produce is a problem (Mulandi, 1998).

Use of resistant varieties has been hampered by the high pathogenic variability of the pathogen (Lashomb & Casagrande, 1981). Cultural control measures such as use of crop rotation cannot be relied upon to control the disease completely (Agrios, 1998). *Streptomyces* are known to produce antibiotics that substantially affect the dynamics of coexisting micro-organisms. In *Streptomyces*, diffusible chemical signals that induce antibiotic synthesis and morphological differentiation include, A-factor and C-factor produced by *Streptomyces griseus*, I-factor by *Streptomyces viridochromogenes*, virginiae butanolides by *Streptomyces virginiae*, IM-2 by *Streptomyces* spp strain FRI-5. Studies done *in vitro* showed that *Streptomyces* spp strains coded 28P, CS35, 14P and CS32 produced antibiotics that inhibited the growth of *Pythium* spp *(*Muiru, 2000). The overall objective of this study was to establish the efficacy of culture filtrates produced by antagonistic *Streptomyces* spp. against late blight of tomatoes under field conditions.

Methodology

Production and assay of antibiotics in liquid culture filtrates
Streptomyces sp. (28P and CS35) isolates were cultured on Czapeks Dox agar, to produce inoculum for liquid fermentation cultures. The test culture filtrates were produced by growing *Streptomyces* isolates 28P and CS35 in glucose-soybean medium consisting of 30g soybean meal, 20g commercial glucose, and 1000ml of distilled water. The cultures were incubated at ambient temperature on a circulatory shaker at 125 rpm for 7 days. Culture filtrates

were harvested by centrifugation and concentrated to 30% by evaporation of water under vacuum using a rotary evaporator. The filtrates were assayed for antibiotic activity using the paper disc method. *Pythium* sp. was used as the test pathogen. Sterilized potato dextrose agar (PDA) cooled to 40^{O}c seeded with 10% (v/v) *Pythium* 10^5 propagules/ml, was dispensed in sterile petri-dishes and allowed to cool. Sterile filter paper discs measuring 9cm diameter dipped in the culture filtrates were placed at equidistant points on the solidified seeded media. Antibiotic activity was determined by the diameter of clear zones of inhibition formed around the paper discs after 48 hours of incubation at ambient temperature.

Effect of antibiotic culture filtrates on late blight under field conditions. Four field experiments were set up at the Upper Kabete field station research farm. The experiments were laid out in a randomized complete block design (RCBD). The treatments were 28P, CS35, 28P + CS35 and chemical standard (Dithane M45) and water as a negative control. Each treatment was replicated four times. The application of the treatments was done 30 days after sowing and an application interval of 10 days was followed. In the first and second experiment, the culture filtrates had not been concentrated. Data was taken one week after the application of the treatment and there after every week. In the third and fourth experiments, the culture filtrates were concentrated by removing 30% of the water.

The effect of combining 28P and CS35 on the efficacy of the culture filtrates was also investigated in the third experiment. In the first and second experiments, the size of each plot was 4m X 3.6m giving rise to 50 plants per plot. Ten plants were tagged per plot and the treatments were applied on these tagged plants. In the third experiment, micro plots measuring 2mx 2m were used and five plants were tagged per plot. Treatments were applied on all the plants in the plot. Data was collected from the tagged plants.

Inoculation and disease assessment. In the first experiment, one plant from the middle of each plot was selected and inoculated to act as a source of inoculum. Natural inoculum was relied upon in all the other experiments. Disease on tagged plants was assessed on weekly basis (7-days interval). Disease incidence was determined as percentage of infected leaves per plant, while disease severity was determined using a 0-9 severity scale where 0 indicates no disease and 9 indicates whole plant infected. At physiological maturity, tomato fruits from each tagged plants were harvested and weighed separately to determine fruit yield. Data was analysed using Genstat® 3.0 statistical software.

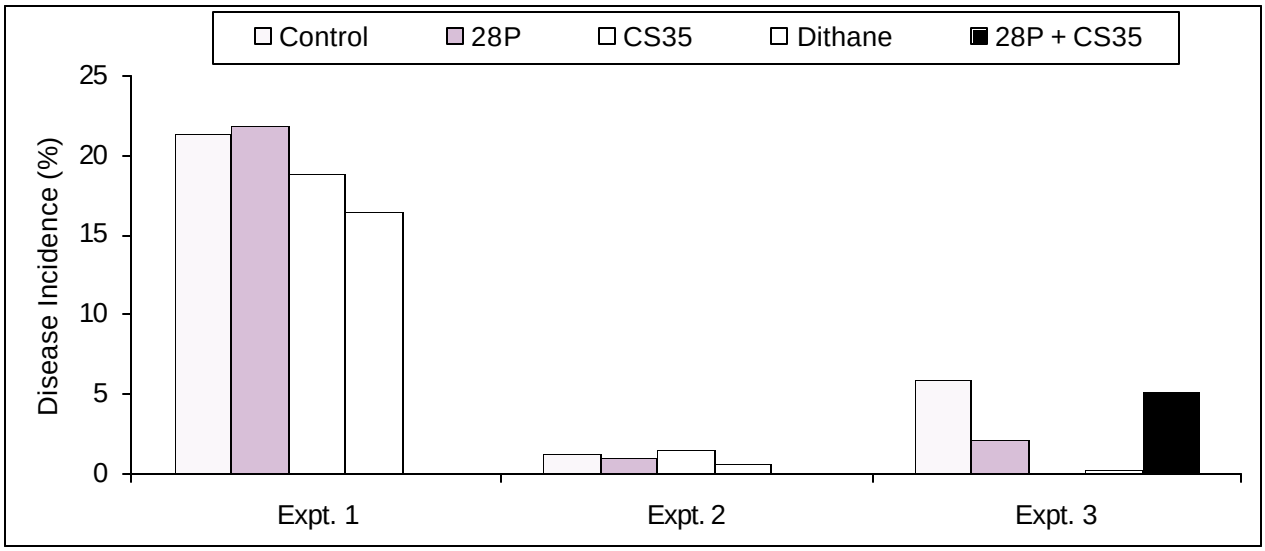
Results

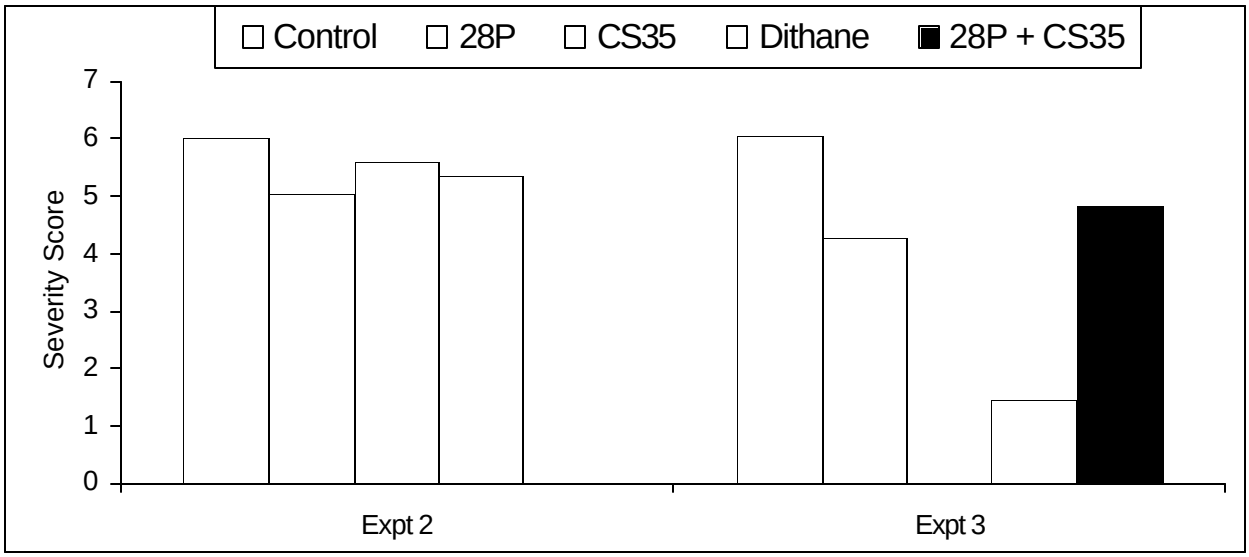
The results showed varied performance in the control of late blight as shown by figures 1, 2 and 3. In experiment 1, the disease pressure was so high resulting in high disease incidences as shown in figure 1. Isolate 28P performed better than isolate CS35 (Fig 1 and Fig 2) and the performance was comparable to that of standard chemical check (Dithane M45). Mixing culture filtrates from the two isolates CS35 and 28P reduced the activity resulting in a higher disease incidence. The various treatments had significant (P =0.05) effect in controlling the late blight as shown in table 1. Dithane M45 had consistently better performance followed by 28P in all the experiments. Plants treated with 28P + CS35 had a mean disease severity of 4.84% while those which were treated with 28P and Dithane M45 had mean disease severity scores of 4.3 and 1.44 respectively compared to 6.03 of the untreated controls (Table 1).

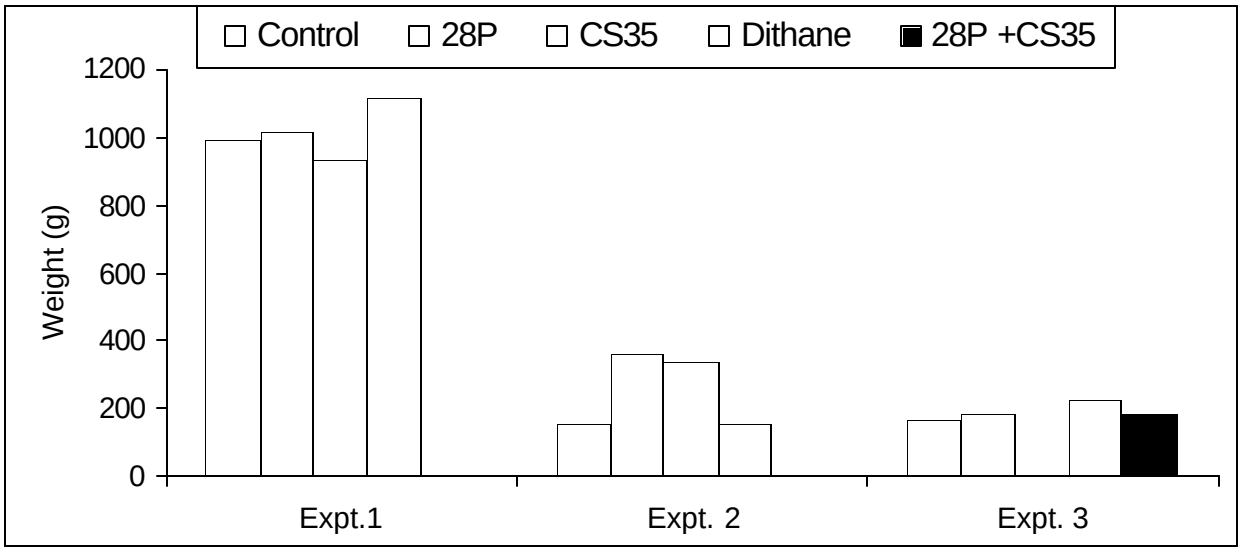
For disease incidence, the tomato plants treated with Dithane M45 had the lowest mean disease incidence of 0.27%, those treated with 28P had 2.09% compared to the control plants which had 5.91%. Plants treated with 28P and Dithane M45 had mean disease incidence values which were not significantly (P=0.05) different from each other. The various treatments had no significant (P=0.05) effect in the yield. Experiment 1 had the highest yield since the disease had set in late in the season.

Discussion

The culture filtrates tested in the management of the late blight of tomatoes showed that they had positive activity against late blight. However, the activity varied with the culture filtrates from isolate 28P giving better performance. Muiru (2000) had characterized the two antibiotics and found that 28P had two active compounds whereas CS35 had only one. Most antibiotics consist of different compounds that vary in activity (Yeo & Hol, 1997).


Control
28P
CS35
Dithane
28P + CS35
Disease Incidence (%)
25
20
15
10
5
0
Expt. 1
Expt. 2
Expt. 3


Control
28P
CS35
Dithane
28P + CS35
Severity Score
7
6
5
4
3
2
1
0
Expt 2
Expt 3



Higher concentrations of the culture filtrates gave a better disease control of late blight compared to unconcentrated culture filtrates. Smith (1985) reported better control of disease with increased concentrations of antibiotics. Crude culture filtrates had poor performance in terms of disease control compared to centrifuged cell free culture filtrates. Mixing different culture filtrates from Streptomyces 28P and CS35 resulted in reduced activity of 28P. Similar findings were reported by Mesters *et al.* (1994). The reduced activity could be due to differences in different active ingredients which reduce the efficacy of each other and different modes of action. The activity of the antibiotics can be enhanced through inclusion of additives such as calcium as has been suggested by Conway *et al.* (1998). Treatments of the tomato plants with the antibiotic culture filtrates and Dithane M45 did not result in significant ($p \leq 0.05$) increase in fruit yield. Yield trait is affected by many factors like disease, genotype, environmental factors and their interaction (Cheah *et al*., 2001).

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