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# CONTROL OF CARNATION FUSARIUM WILT USING ANTIBIOTICS CULTURE FILTRATE FROM *STREPTOMYCES SP.*

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## ABSTRACT

***Streptomyces*** **spp. isolated from soils collected from Kabete, Department of Crop Science, University of Nairobi isolates were screened for antibiosis against *Fusarium oxysporum* f. sp. *dianthi in vitro*. The culture filtrate of isolate 21, one of the antagonistic isolates was tested for activity and found to suppress growth of *F. oxysporum* f.sp *dianthi in vitro*. The culture filtrate was used to treat pathogen inoculated carnation cuttings and it reduced stem discolouration by 62.2% which was comparable to reduction by benlate that reduced stem discolouration by 78.3%. Different concentration levels of the culture filtrate (concentrated filtrate, normal filtrate, half strength filtrate and quarter strength filtrate) all effectively reduced discolouration of the carnation stems by 93.3, 73.1, 68.5 and 63.4%, respectively. Phytotoxicity that led to chlorosis and partial wilting during the first week of treatment was detected in the antibiotic treated plants especially when applied to young and tender cuttings. The chlorosis and partial wilting were significantly reduced when older and well established cuttings were used and when the culture filtrate was diluted to half or quarter strength.**

***Key Words*****: Antibiotics, carnations, Fusarium wilt, *Streptomyces sp.***

## RÉSUMÉ

**Des *Streptomyces spp.* étaient isolies a partir d'echantillons de sol collectés à Kabete, Department des Sciences Agricoles, Université de Nairobi. Les isolats étaient passés au criblage afin de determiner des substances toxiques qui peuvent detruire le *Fusarium oxysporum* f. sp. *dianthi in vitro*. Le filtrat de culture de l'isolat-21, l'un des isolats antagonistes était testé pour son activité. Celui-ci s'est montré capable d'inhiber la croissance de *Fusarium oxysporum* f. *sp. dianthi in vitro*. Le filtrat de culture était utilisé pour traiter les boutures de l'ocillet inoculés avec le pathogène. Ce traitement a reduit le jaunissement des tiges de l'ordre de 62.2%, ce qui ést comparable a la réduction par le benlate (78,3%). Les differents niveaux de concentration du filtrat (filtrat concentrée, filtrat normal, moitié et quant de la teneur du filtrat) ont réduit le jaunissement des tiges de l'ocillet de 93,3, 73,1, 68,5 et 63,4%, respectivement. La phototoxicitée qui a conduit a une chlorose et au flétrissement partiel pendant la premiere semaine du traitement était detectée chez les plantes traitées a l'antibiotique, particulierement sur les boutures jeunes et tendres. La chlorose et le flétrissement partiel étaient réduits significativement lorsque les boutures vieilles et bien etablies étaient utilisées et lorsque la teneur du filtrat de culture était diluté de moiti~e ou de quart.**

***Mots Clés*****: Antibiotiques, Ocillet, Flétrissement de Fusarium, *Streptomyces sp.***

## INTRODUCTION

Fusarium wilt of carnation, caused by *Fusarium oxysporum* Schl. f. sp. *dianthi* (Prill. Del.) Snyder & Hansen, is one of the most important diseases of carnations in the world. It occurs wherever carnations are grown and no *Dianthus* is completely resistant to the two known strains of the pathogen (Fletcher, 1984; Rattink, 1989). Control of the disease is difficult and normally soil fumigation using methylbromide, metham-sodium and soil steaming are the adopted methods for its control. Chemical control is not very effective but benzimidazoles and prochloraz manganese have shown positive responses when applied at the right time (Fletcher, 1984).

This study was undertaken to test the efficacy of an antibiotic culture filtrate from a *Streptomyces sp.* in controlling carnation wilt in the green house. *In vitro* studies had indicated that the culture filtrate suppressed *F. oxysporum* Schl. f. sp. *dianthi*, *Colletotrichum coffeanum* Noack, *Colletotrichum lindemuthiarum* (Sacc. & Magh) Briosis & Car, and *Alternaria crassa* (Sacc.) Rands (Muthomi, 1992). The work was carried out in the greenhouse at Kabete, University of Nairobi.

## MATERIALS AND METHODS

**Preparation of the culture filtrate and carnation plants.** The liquid medium used was Glucose-Soy bean broth (Pridham *et al.*, 1956) sterilized at 121°C for 15 minutes at one bar pressure and cooled to 40°C . The *Actinomycete* suspension used to inoculate the flasks was prepared by flooding a 21-day old *Streptomyces* isolate 21 with sterile distilled water. A flamed wire loop was used to dislodge the *Actinomycete* spores from the colony surface. Using a sterile pipette, 5 ml of the *Streptomyces* suspension was transferred into each flask containing the sterilized medium (Glucose-Soy bean broth) and the flasks were sealed with aluminium foil to prevent contamination. Flasks were incubated at 20 ± 2°C for seven days on a rotary mechanical shaker (65 rpm). After incubation, the fermentation broth was centrifuged at 5000 rpm for 15 minutes. The supernatant was decanted into sterile reagent bottles and stored in the refrigerator at 4°C until required.

Pure cultures of *F. oxysporum* f. sp. *dianthi* isolated from diseased carnations were prepared on potato dextrose agar (PDA) and grown for 14 days. The pure cultures were flooded with sterile distilled water and the surface gently scraped with a sterile glass slide to dislodge the spores. The suspension was filtered through a double layer of cheese cloth to remove the mycelial clumps and the spore concentration determined using a haemacytometer and adjusted to 106 spores $ml^{-1}$.

Healthy spray carnation cuttings were treated by dipping the cut end in captan and were rooted in vermiculite in a rooting chamber for six weeks. The well rooted cuttings were selected and planted in sterilized soil. They were allowed to grow for four weeks after which they were subjected to various treatments with the antibiotic culture filtrate prepared as above. Benlate (0.1%), a systemic fungicide, was used as a check in the experiments.

The well rooted cuttings prepared as above were inoculated with the *F. oxysporum* f. sp. *dianthi* by dipping them in the spore suspension (106 spores $ml^{-1}$) for 15 minutes. The cuttings were subjected to different antibiotic treatments.

Efficacy of isolate 21 antibiotic was tested by comparing disease severity in *F. oxyporium* f.sp. *dianthi* inoculated plants treated with either the antibiotic or benlate. Root cuttings were first dipped into the fusarium spore suspension ($10^6$ spores $ml^{-1}$) for 30 minutes before treating for 15 minutes with the antibiotic or benlate. There were two controls, cuttings dipped in the fusarium spore suspension and cuttings dipped in sterilized distilled water. Plants used in this experiment were allowed to grow for four weeks in sterilized soil after rooting. In the second experiment, the same treatments were used but the inoculated plants were first planted in non-inoculated soil and allowed to grow for seven days to allow the establishment of the pathogen within the carnation plants. After seven days the plants were uprooted and excess soil around the roots washed off in running water. The plants were then dipped in the antibiotic culture filtrate or benlate (0.1%) as above. The plants used for this second experiment were younger than those in experiment 1.

In both experiments, the plants were grown in 6-cm diameter polythene sleeves containing previously sterilized soil. In experiment 1, each sleeve contained three plants, and was replicated

four times while in the second experiment, each treatment had four polythene sleeves of three plants each and was replicated four times. The sleeves were arranged in the green house in a completely randomized design. The plants were allowed to grow up to the time the first disease symptoms appeared. All the plants in each treatment were then uprooted, washed in running water and sectioned longitudinally. The extent of vascular discolouration was recorded.

**Effect of different concentrations of antibiotic culture filtrates on the level of control of fusarium wilt pathogen of carnation.** Four antibiotic concentrations were prepared; (i) normal concentrate (supernatant); (ii) concentrate filtrate obtained by reducing the supernatant from 300 ml to 200 ml using a rotary vacuum evaporator; (iii) half-strength filtrate created by adding an equal volume of sterile distilled water to the filtrate; and (iv) quarter strength filtrate prepared by adding three parts water to normal culture filtrate.

Seedlings treated with the different antibiotic concentrations were inoculated by dipping in a spore suspension ($10^6$ spores $ml^{-1}$) for 30 minutes. The control root cuttings were dipped for 15 minutes in the spore suspension and planted in non-inoculated soil. In the second control they were dipped in sterile distilled water and planted in non-inoculated soil. The plants were planted in polythene sleeves each containing 3 plants and were arranged in a completely randomized block design with four replicates. The cuttings were allowed to grow for 8 weeks being watered daily before assessment for disease severity. Longitudinal sectioning of the stems was carried out and the length and percent vascular discolouration recorded.

**Assessment of Fusarium wilt of carnation.** The extent of vascular discolouration, determined by longitudinal sectioning of the sampled stems, was expressed as a percentage of the total stem length. Plants were assessed for disease severity from the time of establishment up to when the plants were 13 weeks old and included apparently healthy plants to severely wilted and deformed plants. Disease severity was given a rating of 0-10, discolouration with 0 = healthy (no discolouration), 1 = 1–9%, and 10 = 90–100% discolouration.

## RESULTS

**Efficacy of antibiotic culture filtrates of *Actinomycete* isolate 21.** Stem discolouration measurements showed that antibiotic treatment reduced the amount of disease on the carnation plants. The reduction was comparable to that achieved by benlate treatment used as a check. Disease control was observed as delayed expression of the typical disease symptoms and a reduction in the vascular discolouration. In Experiment 2 the percentage number of plants showing external disease symptoms was lower in plants treated with antibiotic culture filtrate than in those treated with benlate (Table 1). The antibiotic and benlate treatments reduced stem discolouration by 62.2 and 78.3%, respectively, compared to the mean discolouration in the nontreated inoculated control. There were no significant differences between the mean stem discolouration in benlate treated and antibiotic treated plants. It was observed that the antibiotic treated plants had a poor take-off after planting. In Experiment 2 where the plants were young and tender, there was high mortality one week after treatment, but in Experiment 1 where the plants were old and stronger, no mortality was observed in any of the treatments. The negative effect of antibiotic treatment was reflected in the significant reduction in stem lengths of the antibiotic treated plants in Experiment 2 but no negative effects were observed in Experiment 1 (Table 2).

**Effect of concentrations of antibiotic culture filtrate on the level of controls of Fusarium wilt pathogen of carnation.** There were no significant differences among the mean stem discolouration of plants treated with different antibiotic culture filtrate concentrations (Table 3). However, significant differences ($P \leq 0.05$) were observed between the stem discolouration of the non-treated inoculated control and the main stem discolouration of each of the other treatments. Stem discolouration was reduced by 93.3, 73.1, 68.5 and 63.4% in the concentrated, normal, half-strength and quarter strength filtrates, respectively. Plant mortality decreased when low antibiotic concentrations were used (Table 3).

## DISCUSSION

The *in vivo* studies showed that antibiotic treatment of carnation plants inoculated with *F. oxysporum*

TABLE 1. Symptom expression in carnation plants inoculated with Fusarium wilt pathogen and treated with Streptomyces antibiotic culture filtrate and benlate

| Symptom expression | Antibiotic inoculated | Benlate inoculated | Non-treated with antibiotic, inoculated with pathogen | Non-treated with antibiotic, non-inoculated with pathogen |
|---|---|---|---|---|
| **Experiment 1** | | | | |
| Mean percentage stem discolouration (mm) | 8.94[ab] | 5.12[a] | 23.66[b] | 2.23[a] |
| % plants showing external symptons | 0.00 | 0.00 | 27.27 | 0.00 |
| l.s.d. (0.05) = 14.74 | | | | |
| **Experiment 2** | | | | |
| Mean percentage stem discolouration (mm) | 11.01[ab] | 12.69[ab] | 21.97[b] | 0.00[a] |
| % plants showing external symptoms | 0.00 | 40.00 | 27.59 | 0.00 |
| l.s.d. (0.05) = 12.75 | | | | |

Means in the same row followed by the same letter are not significantly different at 5% level of probability.

TABLE 2. Mean stem length (mm) of carnation plants treated with Streptomyces antibiotic culture filtrate and benlate

| Treatment | Stem length (mm) | Range (mm) |
|---|---|---|
| **Experiment 1** | | |
| Antibiotic treated and inoculated with pathogen | 93.33[a] | 80.00–107.50 |
| Benlate treated and inoculated with pathogen | 98.13[a] | 85.00–107.50 |
| Non-treated and inoculated with pathogen | 90.00[a] | 81.67–110.00 |
| Non-treated and non-inoculated with pathogen | 97.08[a] | 90.00–103.33 |
| **Experiment 2** | | |
| Antibiotic treated and inoculated with pathogen | 54.13[b] | 52.14–56.11 |
| Benlated treated and inoculatedwith pathogen | 76.35[a] | 68.89–82.22 |
| Non-treated and inoculatedwith pathogen | 59.38[b] | 58.75–60.00 |
| Non-treated and non-inoculated with pathogen | 82.03[a] | 66.43–92.50 |
| l.s.d. (0.05) = 14.58 | | |

Means followed by different letters down the column of experiment 1 and experiment 2 are significantly different at 5% level.

TABLE 3. Mean percentage vascular discolouration and stem length (mm) of carnation plants inoculated with Fusarium wilt and treated with different concentrations of *Streptomyces sp.* culture filtrate from isolate 21

| Treatment | Mean stem length (mm) | Mean vascular | % mortality discolouration |
|---|---|---|---|
| Concentrated antibiotic filtrate-inoculated with pathogen | 79.17[a] | 1.43[a] | 41.67 |
| Normal antibiotic filtrate-inoculated with pathogen | 68.13[a] | 5.77[a] | 41.67 |
| 1/2 strength antibiotic filtrate-inoculated with pathogen | 83.84[a] | 6.75[a] | 16.67 |
| 1/4 strength antibiotic filtrate-inoculated with pathogen | 70.00[a] | 7.84[a] | 25.0 |
| Non-treated with antibiotic filtrate and inoculated with pathogen | 70.21[a] | 21.43[b] | 16.67 |
| Non-treated with antibiotic filtrate and uninoculated with pathogen | 92.29[a] | 0.57[a] | 8.33 |
| l.s.d. (0.01) = 14.88 | | | |

Means followed by the same letter down the column are not significantly different at 1% level.

f. sp. *dianthi* both delayed symptom expression and reduced disease severity. The reduction in disease severity was comparable to that achieved by benlate, a broad spectrum systemic fungicide. The effectiveness of crude antibiotics in controlling plant diseases had been reported by other investigators (Pridham *et al.*, 1956; Padmanabhan and Kameswar, 1966; Loeffler *et al.*, 1986 and Mckeen *et al.*, 1986). Padmanabhan and Kameswar (1966) compared the effectiveness of crude mycelial extracts from a species of *Streptomyces* with that of well known antifungal antibiotics, Antimycin-A, Blastimycin, Hamycin, Thioluitin, Blasticidin S, Kasumin and Aureofungin. The extract was found to be as effective as Kasumin, Blasticidin S and Copper and Mercury fungicides in controlling *Piricularia oryzae* Cav. in the field.

In this study, phytotoxic effects were observed when young and tender carnation cuttings were treated with the antibiotic culture filtrates. Phytotoxicity was observed as general chlorosis, prolonged partial wilting which in some cases resulted in high mortality. However, the antibiotic had only slight adverse effects when applied on older plants. Phytotoxicity to young tender cuttings was reduced by diluting the antibiotic culture filtrates without any significant reduction in the effectiveness in control of the disease. Phytotoxic effects of antibiotics have been observed by other workers (Gottlieb *et al.*, 1950; Gregory *et al.*, 1952; Stessel, 1953); Pridham *et al.* (1956), Misato *et al.* (1977). Loeffler *et al.* (1986) observed phytotoxic effects on rice leaves sprayed with higher concentrations of Blasticidin S. The effective concentration of the antibiotic is usually 10–20 μg $ml^{-1}$. The phytotoxic effects of Blasticidin S on rice leaves was influenced by the frequency of application, application time, rice variety, atmosphere conditions such as temperature and moisture, soil type and fertilizer used.

Currently, none of the known control measures is completely effective against Fusarium wilt of carnation. Therefore, more research on antibiotics might result in a remedy for this disease. Antibiotics can be used to eliminate inocula in cuttings since cuttings are the most important primary sources of the pathogen. It can be concluded from this study that antibiotics from the right strain of *Streptomyces* administered as a culture filtrate can effectively reduce wilt of carnation caused by *F. oxysporum* f. sp. *dianthi*. Phytotoxic effects observed can be reduced by diluting the culture filtrate.

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