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Use of *Actinomadura* AC 170 Antibiotics in the Treatment of Some Cultivated Plant Diseases

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ABSTRACT

The influence of antibiotics of *Actinomadura* AC170 (*Actinomycetales*) on the expression of chocolate spot diseases and the *Ascochyta fabae* infection of broad bean and on the *Fusarium* wilt of flax was studied. The antibiotics were produced on solid medium GYEA (glucose-yeast extract-agar), extracted with *n*-butanol and purified on column of Sephadex LH 20 and by preparative thin-layer chromatography. A HPLC analysis allowed to obtain two antibiotics noted 170A and 170B. These antibiotics reduced the foliar diseases but 170A was the most effective. However, these antibiotics did not cause any reduction of the *Fusarium* wilt of flax nor of the population of *F. o. lini* in the soil. 170A reduced the density of fungi only with large amount and in sterilized sandy soil. The antibiotics of *Actinomadura* did not show any inhibitory effect, even with large amount, on the growth of plants.

Key Words: *Actinomadura* sp., antibiotics, chemical control, fungal diseases.

INTRODUCTION

The actinomycetes are positive Gram mycelial bacteria. They are well-known for their production of many antibiotics. The agronomic interest of these bacteria was already cited by Dommergues et Mangenot (1970), Lechevalier (1981) and Goodfellow and Willliams. (1983) on the recycling of the organic matter in the soil, on the symbiotic fixing of nitrogen by *Frankia* genera, and especially on their antagonistic properties which makes them ready to be used in the biological fight against the plant diseases. In this context, Merrouche (2000) showed the interest of thiolutine antibiotic, secreted by *Saccharothrix algeriensis* SA233 in the fight against *Fusarium* wilt of some cultivated plants.

In investigation, we studied another strain of actinomycete, *Actinomadura* sp. AC170, in order to isolate its antibiotics and to test its efficacy against chocolate spot disease, *Ascochyta fabea* infection of broad bean, and *Fusarium* wilt of flax.

MATERIALS AND METHODS

Actinomadura sp. AC 170 was isolated by Boudjella (1994) from Saharan soil. Because the inhbility of Strain AC170 to growth in liquid media, the antibiotics production was done on solid medium, GYEA (Glucose-Yeast Extract-Agar) (Athaley *et al.*, 1981). The antibiotics were extracted by *n*-butanol and then purified on sephadex LH20 column, after that on a preparative thin layer chromatography (silica gel GF254). The purity of products was checked by HPLC (column C18, gradient methanol water: 20 to 100% during 30 min).

Antibiotics efficacies were tested against chocolate spot disease due to *Botrytis fabae* and *Ascochyta fabae* infection of broad bean and on the *Fusarium* wilt of the flax caused by *Fusarium oxysporum* f. sp. *lini*. The plants were sprayed by 1.2 ml of the pathogenic agents' solution per plant. The concentrations were 4×10^5 CFU ml^{-1} or *Botrytis fabae*, and 4×10^4 CFU ml^{-1} for *Ascochyta fabae*. In the case of chocolate spot disease and the *A. fabae*, the antibiotics were applied on young plants (phase of 3 leaves) in proportion of 1.2 mg of antibiotics per plant. The application of these antibiotics was either carried out at the same time of infection of the healthy plants, or on the plants already diseased, or 4 days before of infection (as prevention) on healthy plants. Thirty plants were considered by test. In the case of *Fusarium* wilt of flax, 5 mg of antibiotics was added to 100 g of soil before sowing. *F. o. lini* was transmitted in proportion of 5×10^4 CFU g^{-1} soil.

Also, the effect of antibiotics at 5, 15 and 30 mg/100 g of soil was tested on *F. o. lini* population inoculated in clayed and sandy soils (sterile or not sterile).

The effect of 170A antibiotic on the growth of plants was evaluated at 5 mg/ml. This antibiotic was pulverized on leaves of broad bean, lentil, chickpea, flax, and some weeds (*Avena sterilis*, *Vicia sativa*, *Vicia sicula* and *Dactylis glomerata*) at 0.6 ml per plant (3 mg per plant).

RESULTS AND DISCUSSION

Isolation and purification of *Actinomadura* AC 170 antibiotics

The antibiotics were produced on GYEA medium and extracted by *n*-butanol. The crude butanolic extract, which was submitted to a separation on sephadex LH20 column, gave 6 fractions, F1 to F6. Only F3 and F4 fractions had proved to be active against *Bacillus subtilis* and *Mucor ramannianus* (germs test). The F3 and F4 fractions were separated on preparative thick layer chromatography. Each fraction was composed of two products: 170A and 170B and third one called 170BJ in the case of F4. Sephadex column could eliminate lot of impurities, but it cannot separate antibiotics from each other. They were separated on thick layer chromatography. The HPLC analysis revealed that 170A was constituted of a single product, 170BJ of two products and 170B of a complex of products.

Antibiotics effect on foliar plant diseases

In the case of chocolate spot disease, the spraying of plants with antibiotics, just after an infection of broad bean by *Botrytis fabae*, showed that 170A prevented totally the disease to manifest (in comparison to control without antibiotics, where 94% of plants were diseased). The reduction of disease was of 72% for 170AB (a mixture of 50% 170A and 50% 170B) and 63.3% for 170B (figure 1.1). The application of 170A on plants already infected inhibited completely the evolution of the disease (figure 1.2) and when it is used 4 days before the infection by *B. fabae*, it reduced the disease 53.3% (Figure 1.3). In the case of *A. fabae*, infection of broad bean, 170A reduced the disease with 33.2% and 170B with 17.3% in comparison to the control (Figure 1.4).

Antibiotic effects on *Fusarium* wilt of flax and *F. o. lini*

At the dose of 5 mg for 100 g clayey soil, the antibiotics 170A, 170B and 170AB did not prove any reduction of disease in comparison to the plants without antibiotics (Figure 2).

Antibiotics 170A, 170B and 170AB at 5 and 15 mg/100 g of non sterilized soils did not reduce the *F. o. lini* population neither in clay soil nor in sandy one (Figure 3). However at 30 mg/100 g of sterilized clay soil, no reduction was observed, but at the same concentration in a sterile sandy soil, *F. o. lini* population was reduced about 5 times, in comparison to the control without antibiotics (Figure 4)

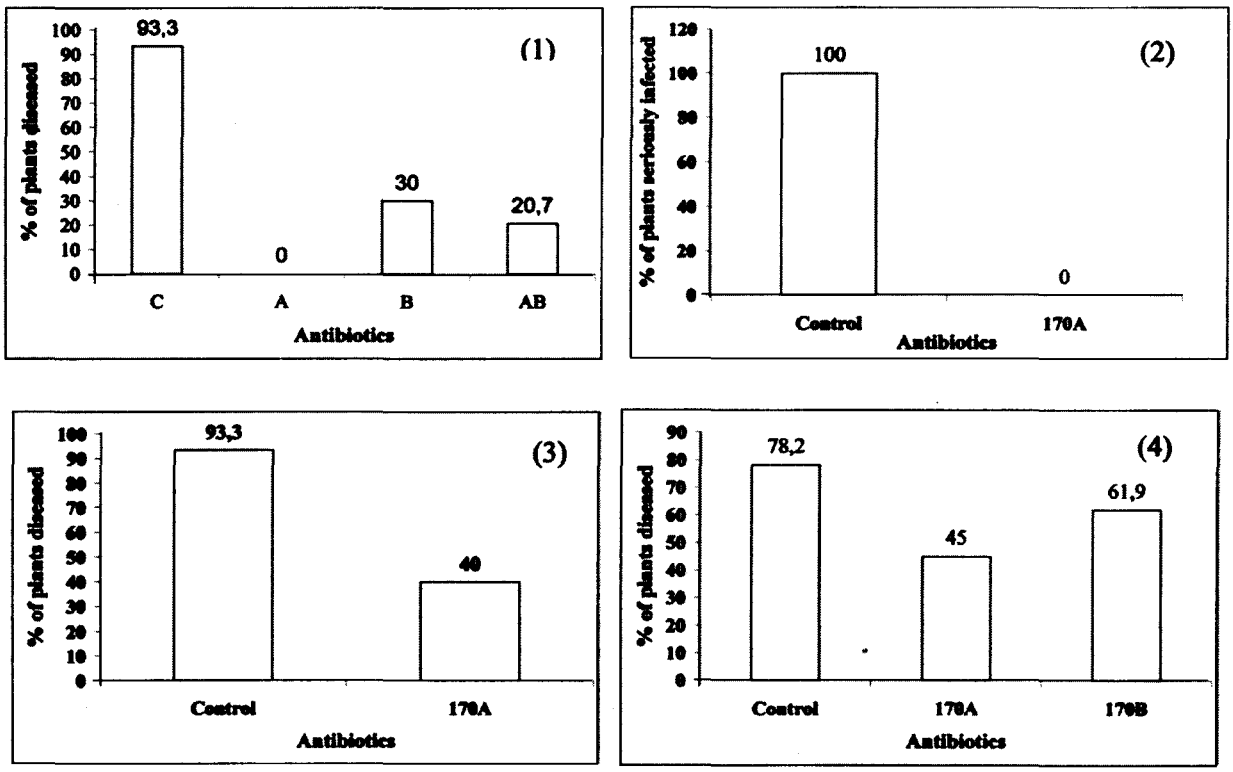

Fig. (1): Effects of *Actinomadura* AC170 antibiotics on: 1. the expression of chocolate spot disease; 2. the evolution of chocolate spot disease; 3. protection of healthy plants from chocolate spot disease; 4. the expression of *Aschochyta fabae* infection of broad bean.
A = 170A; B = 170B; AB = 170A (50%) +170B (50%); C = control.

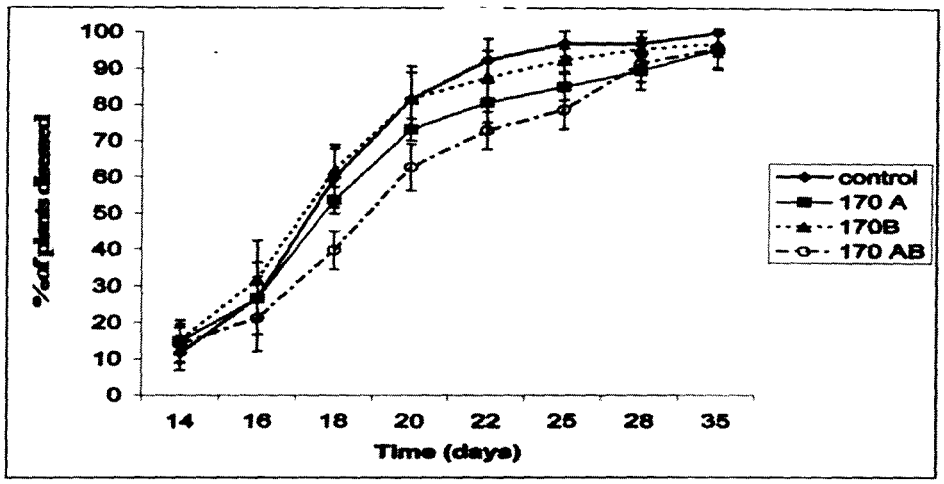

Fig. (2): Effects of the *Actinomadura* AC170 antibiotics on the expression of *Fusarium* wilt of flax in clay soil inoculated by *F. o. lini.*

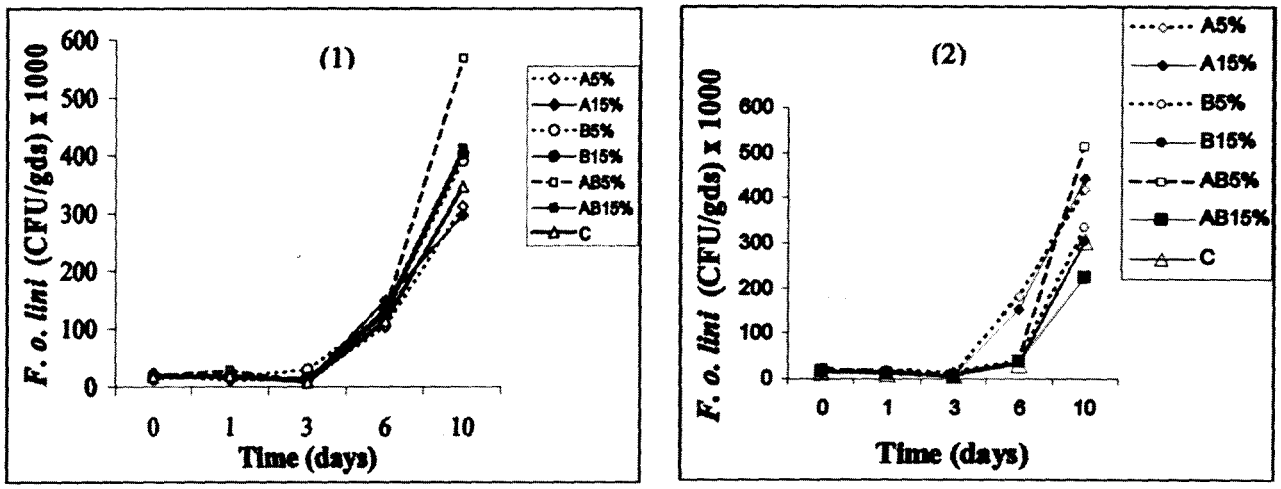

Fig. (3): Effects of antibiotics produced by *Actinomadura* AC170 on *F. o. lini* in soils.
1 = not sterilized clay soil; 2 = not sterilized sandy soil. A, B, AB = *Actinomadura* AC 170 antibiotics; C = control. CFU = colonies forming unite; gds = gramme of dry soil.

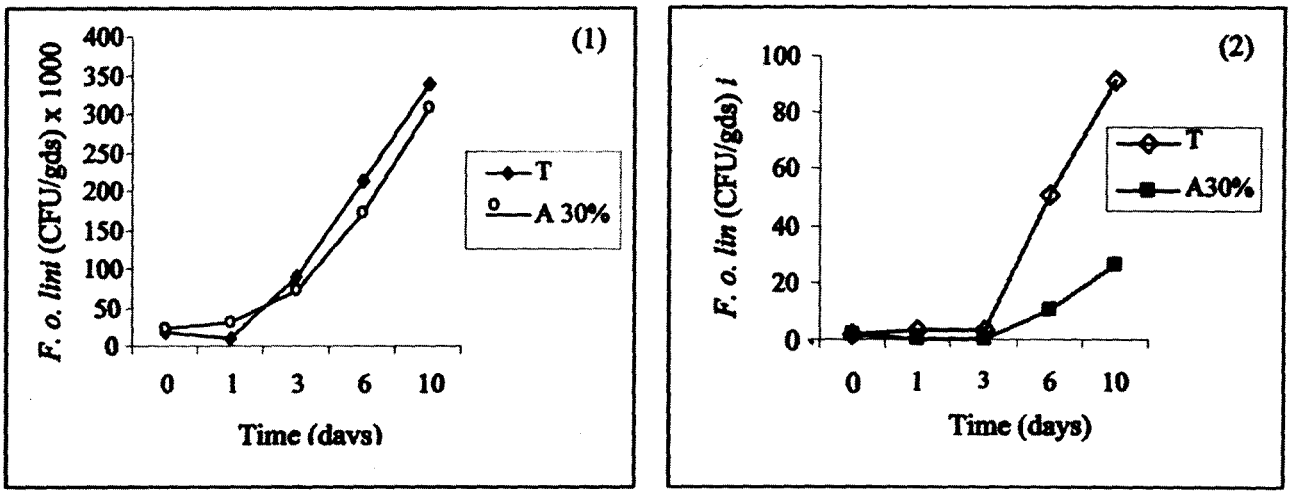

Fig. (4): Effects of *Actinomadura* AC170 antibiotics on *F. o. lini* in sterilized soils.
1 = clay soil; 2 = sandy soil; CFU = colonies forming unit; gds = gramme of dry soil.

Antibiotic effects on growth of plants

The 170A antibiotic used at 3 mg per plant did not affect the growth of cultivated plants (broad bean, lentil, chickpea, flax) and weeds (*Avena sterilis*, *Vicia sativa*, *Vicia sicula* and *Dactylis glomerata*).

Actinomadura AC170 was the most powerful strain among hundred of strains isolated in our laboratory, which belong to various genera and species (Sabaou *et al.*, 1998). The butanolic extracts contain the complex 170A and 170B antibiotics. These antibiotics can be obtained easily by a simple extraction by *n*-butanol, and purification by chromatography on Sephadex LH20 column, which eliminate lot of impurities, and on a thick layer chromatography.

170A and 170B had an aromatic polycyclic nature and their molecular weights were about 280 and 327 according to Mostefaoui (1996) and Badji (2006).

Among the antibiotics used against diseases due to *B. fabae* and *A. fabea*, 170A revealed a high efficacy; it was better than 170B and the mixture 170AB. It showed its efficacy in controlling the chocolate spot disease (it prevented expression of the disease, inhibited its evolution and protected healthy plants) without having any side-effect on the growth, colour or the appearance of the broad bean plants. Maufras(1985) noticed that spraying plants with a mixed product of the benzimidazoles and thiocarbamates reduced chocolate spot disease, but it remained ineffective during serious attacks. Accoding to Harrison (1984) and Fitt *et al.*, (1986), spraying of benomyl or vinclozoline showed a certain efficacy against broad bean disease caused by *B. fabae*. Vinceli and Lorbeer (1989) and Khöl *et al.*, (2003) found that the vinclozoline and other fungicides reduced from 50 to 60% of the foliar spot disease of onion caused by *Botrytis cinerea* and *B. squamosa*. Throughout the results, 170A antibiotic proved to be among the most effective molecules in the treatment of chocolate spot disease.

Concerning a broad bean infection caused by *A. fabae*, the antibiotic 170A seemed to be more effective than 170B. However, this efficacy was lesser than the one observed with chocolate spot disease.

The influence of *Actinomadura* AC170 antibiotics on *Fusarium* wilt and on the evolution of *F. o. lini* in the soils, gave rather deceiving results. These antibiotics did not show any aptitude to reduce the disease. Even with strong concentration, the *F. o. lini* population was not decreased in the two soils (clay and sandy), except in the case of sterilized sandy soil. The results obtained suggested that the antibiotic 170A was fixed by the clay or degraded by the soil flora. Thus, the antibiotic 170A would be cationic. Conversely, the results obtained by Merrouche (2000) revealed that the thiolutine, produced by *Saccharothrix algeriensis,* was too effective against the *Fusarium* wilt of flax and other cultivated plants (*F. o. lini* strain, the variety of flax and the soil used were the same elements used in this study).

It could be concluded that the strain *Actinomadura* AC170 was interesting for its secreted bioactive products. Its antibiotics could be obtained by production on GYEA medium, extraction by the *n*-butanol, purification on sephadex LH20 column and thick layer chromatography. These antibiotics are effective against foliar disease caused by fungi especially chocolate spot disease of broad bean. Among these antibiotics, 170A proved to be the most powerful. This interest was enhanced by the fact that the molecules do not present any toxicity on the broad bean and other plants, even with high doses.

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الملخص العربى

إستعمال البكتيريا الهيفية *Actinomadura* sp. AC 170 لمكافحة بعض أمراض النبتات المزروعة

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يتناول هذه العمل دراسة تأثير المضادات الحيوية لسلالة من البكتيريا الهيفية (الاكتينوميسات): *Actinomadura* sp. AC 170 على أمراض النباتات التالية : مرضي لطخة الشكولاطة (chocolate spot) والفحومة (*Ascochyta fabae* infection) للفول ومرض الذبول الوعائي لنبات الكتان (*Fusarium* wilt). تم إنتاج المضادات الحيوية للسلالة *Actinomadura* AC 170 على وسط غذائي صلب GYEA واستخلاصها بـــ *n*-بوتانول (*n*-butanol) ثم تنقيتها بالترشيح الهلامي بـــ Séphadex LH 20 وعلى صفائح السيليس GF254. بين التحليل بالكروماتوغرفيا السائلة فائقة التجلية (HPLC) في الطور المعكوس (عمود C18) بأن المضادات الحيوية ١٧٠A، ١٧٠B و ١٧٠BJ المتحصل عليها تكون سواء" معزولة (١٧٠A) أو في حالة معقد يتكون من جزيئتين (١٧٠BJ) أو معقد يتكون من عدة جزيئات (١٧٠B). درس تأثير معقدات المضادات الحيوية التالية: ١٧٠A، ١٧٠B ، ١٧٠AB (خليط)، على الأمراض النباتية المذكورة سابقا. فالمضاد الحيوي ١٧٠A هو الأكثر فعالية ضد مرض لطخة الشكولاطة المسبب من طرف *Botrytis fabae* اذ يوفر حماية كلية عند استعماله مباشرة بعد اجراء العدوى على أوراق نبات الفول السليمة، كما يثبط كليا تطور هذا المرض عند استعماله على نباتات مريضة. رش نباتات الفول السليمة بهذا المضاد الحيوي (١٧٠ A) اربعة ايام قبل اجراء العدوى (بغرض الحماية) يوفر حماية قدرها ٥٣,٣% . أما مرض الفحومة المسبب من طرف *Ascochyta fabae* فانه ينخفض بنسبة ٣٣,٢%. المضاد الحيوى ١٧٠B يخفض بدوره مرض لطخة الشكولاطة بنسبة ٦٣,٣% و ١٧٠AB بنسبة ٧٢ %. من جهة أخرى المضادات الحيوية ١٧٠A ، ١٧٠B و ١٧٠AB لا تحدث أي انخفاض في مرض الذبول الوعائي لنبات الكتان عند استعمالها بتركيز ٥ ملجم في ١٠٠غ تربة طينية. هذه المضادات الحيوية ليس لها أي تأثير على عشيرة الفطر *F. o. lini* عند استعمالها بتراكيز ٥ و ١٥ ملجم في ١٠٠ جم تربة طينية أو رملية. لا تنخفض كثافة الفطر الا عند استعمال المضاد الحيوي 170A بتركيز ٣٠ ملجم في ١٠٠ جم تربة رملية معقمة فقط ، مما يؤدي بنا إلى طرح إمكانية ادمصاص هذا المضاد الحيوي من طرف حبيبات الطين أو احتمال تفكيكه من طرف الفلورة الدقيقة للتربة. بهذا تتضح أهمية المضاد الحيوي ١٧٠A في معالجة الأمراض الفطرية الهوائية مثل لطخة الشكولاته و مرض الفحومة للفول.