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FORMULATION OF STREPTOMYCES ANTAGONIST WITH INERT AND ORGANIC CHARGES AGAINST MYCOSPHAERELLA FOOT ROT AND BLIGHT

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Résumé

Le manque à la levée et la pourriture du pied (foot rot) sont les principaux dégâts occasionnés sur pois aux premiers stades de développement de la plante. Ces symptômes sont provoqués par My*cosphaerella pinodes*., agent de l'anthracnose du pois.

Des souches d'Actinomycètes isolés de la rhizosphère, ont été testées pour leurs activités antagonistes sur l'agent de l'anthracnose. La souche de *Streptomyces* (St7c5) s'est montrée très performante pour son une activité antagoniste vis-à-vis de *Mycosphaerella* pinodes. Des tests in vivo, nous ont permis de noter ces performances aussi bien au niveau du taux de germination qu'au niveau de la croissance de la plante. La comparaison des différents charges à permis de retenir l'association talc- Streptomyces comme étant la formulation la plus efficace pour une meilleure protection de la graine mais aussi la plantule contre les symptômes de foot rot. D'autre part, cette formulation a permis de réduire le taux de pourriture de collet à 5% comparativement au témoin (30.5%).

Donc, Sur la base de ces résultats, nous pouvons recommander l'utilisation de cette formulation comme un moyen de protection contre l'anthracnose à tous les stades de végétation.

Mots clés : *formulation, Streptomyces, Antagonisme, Mycosphaerella pinodes, Foot rot, anthracnose.*

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Abstract

Peas is highly susceptible to preemergence damping off, caused by *Mycosphaerella pinodes* in western Algerian regions. Rhizosphere actinomycetes which were antagonistic this pathogen was isolated from chellif soils. An isolate of *Streptomyces* (St7c5) provided superior seed protection. Increased in both the germination and plant growth were recorded following treatment of seeds with *Streptomyces* formulated with inert or organic charge when compared to control. Application of the antagonist agent resulted in a significant reduction of *Mycosphaerella* foot rot to 5% compared to untreated seeds (30.5%).

Application of *Streptomyces* resulted in a significant reduction of blight symptoms when compared to the control or when treated with talc alone. Hence, the talc formulation of *Streptomyces* agent can be recommended as one of the crop strategies for the management of foot rotting and blight caused by *Mycosphaerella pinodes*.

Keywords: *Talc formulation, Streptomyces, Antagonism, Mycosphaerella pinodes, Foot rot, Ascochyta blight.*

ملخص

يعد التعفن التاجي من اخطر امراض التي تصيب محصول الجلبان في العديد من دول الحوض الابيض المتوسط من بينها الجزائر بسبب الفطر MYCOSPHAERELLA PINODES

ان الدراسة العوامل المثبطة لهذا المرض اضهرت وجود جذ مات علي مستوى الجذور تصنف من بين Actinomycetesخلال هذا الدراسة تم عزل سلالة من جنسي Streptomyces تم التعرف عليها بواسطة اختبارات مرفولوجية .فيزيولوجية وبيوكيماوية اما التجارب الميدانية فقد بينت مادای تاثيرها على تخفيف من حدة التعفن عند البذور التى تم حقنها بواسطة:Streptomyces بواسطة حوامل عضوية او معدنية , وكانت هذه التخفيضات تصل الى اقل من خمسة بلمئة مقارنة بتلك التي لم يتم معلجتها

الكلمات المفتاحية : *تثبيط الحوامل, , Streptomyces, Actinomycetes, Ascochyta pinodes, التعفن التاجي/*

The most serious disease of processing peas in Algeria is *Mycosphaerella pinodes* (**BOUZNAD, 1988**). Despite progress in the development of resistant germplasm in recent years (**TIVOLI, 1998**), there are no commercial cultivars with resistances to all races.

Certain fungicides are used to control pea anthracnosis. However, there are not always economically practical. Furthermore, general, concern about the adverse effects of the pesticide on environment pollution and the quality makes it desirable to find alternative control measures (BEALE and PITT, 1990; WALTER *et al*.1995, THIRUP.L, *et al*, .2003).

Mycosphaerella pinodes can affect the emergence of the seed and cause foot rot in early stage more than necrosis on leaf and aerial parts of the plant.

Formulation with certain bacteria and fungi has been reported to control several soils borne disease (WALTER e*t al.*1995, THIRUP.L, *et al*, .2003).

Although *Streptomyces* antagonist has been extensively studied against root diseases of several fields, its usage for the management of *Mycosphaerella* disease is very limited WALKER *et al*,1998; WALTER *et al*.1995).

Therefore, the present study was aimed to evaluate the potential of seed formulation with various organic and inert carriers have also been used ,upon which biocontrol agent are grown before to control of the emergence and foot rot and plant growth by evaluating both the stem and root length.

MATERIALS AND METHODS

Field collections and isolation of *Streptomyces*

In 2005, *Streptomyces* were isolated from rhizosphere of healthy appearing pea plants grown in field soils throughout 10 locations in Chlef region in west Algeria.

Characterization of active *Streptomyces*

The *Streptomyces* isolates are screened for activity against *M.pinodes* in dual layer culture isolates. Among them, the isolate St7c5 was the most efficient in inhibition the growth on nutrient media of *M. pinodes* isolated from pea seed. A stock culture of this isolate was maintained in sterile deionized water at 5C for one month days. This *Streptomyces* isolate was identified by cell morphology, by noting reactions to Gram stain, and by using biochemical tests like casein hydrolysis, aerobic growth, catalase production, starch hydrolysis and acid production from mannitol (Table 1).

Activity studies

The activity of St7c5 was tested in dual cultures for isolate Mp1bs of *Mycosphaerella pinodes* isolated from pea seed.

Dual cultures were prepared on PDA (Potato Dextrose Agar) by streaking a 0.01ml sample of *Streptomyces* isolate suspension 5mm from the edge of each of four 9cm culture plates.

Table1: Cultural and morphological characteristics of St7c5 organism on 7 media.

Medium	Growth	Aerial mycelium	Spore color
Czapeck Dox agar	Moderate growth with white color	Very abundant	White color
Peptone yeast extract agar-iron	Brown color; moderate growth	abundant	Abundant with white color
yeast malt extract agar	Well abundant with creamy color		Moderate with white color
Nutrient agar	Absent	Absent	Absent
PDA	Creamy colonies with moderate growth	Absent to very poor	Very poor
King B	Moderate, with creamy color	Absent to very poor	Poor
Sabouraud	Very poor growth	Very poor	Very poor

After 3 days at 22°C, a 5mm agar disk of *M.pinodes* (Mpbs) was placed 5cm opposite the *Streptomyces* streak (ROTHROCK and GOTTLIEB**,** 1981). An agar disk of the same fungus placed on culture plate without the bacteria served as a control.

After 7 days at 22°C, inhibition was recorded if the radial growth of the test fungus was greater on the control on the *Streptomyces* – fungus plate.

At that time, $5mm^2$ pieced of mycelium cut from the colony margin was transferred to fresh medium and radial growth was recorded 7days at 22°C to determine if inhibition was fungistatic or fungicidal.

Greenhouse studies

Pea seeds were tested with suspension of St7c5. The experiment was conduced using pea seeds. Lots with 96% germination, was determined by germination test on moist filter paper at nearly 100%RH for 5 days at 22°C.

Hundred seeds were dipped into *streptomyces* suspension concentrated at 10^8cfu, then coated with a 5g of charges (pectin, starch and talc).

Twenty five seed per replicate were sprayed with the fungus isolate before placed in Petri dish for the evaluation of the emergence.

For evaluating the foot rot symptoms due to *M. pinodes*, 100 treated seeds as for germination tests were planted in

15cm diameter sterilized sand/clay soil. The pots were plated at 22°C temperature with 80% RH.

21 days after planting, foot rot symptoms root and stem length was recorded. Three replicates were maintained for each test.

Statistical analysis

The experimental data were analyzed statistically by one way ANOVA using STATITF software. The Newmann-Keuls tests were used to separates groups when ANOVA were significant at P≤0.05.

RESULTS

Isolation and Activity of isolates Actinomycetes

Among the 80 Actinomycetes isolates that were obtained from the rhizosphere against the *M. pinodes,* most of them suppressed the growth of the fungi. The isolate that gave the greatest percentage of inhibition was named St7c5 which inhibited the radial growth to 80% compared to the control.

Characterization of the active *Streptomyces* antagonist

After reisolation of the St7c5 from inhibited colonies on fresh PDA, the isolate obtained was identified. The isolate was Gram +, forming spores chain and was positive for casein hydrolysis, catalase production, starch hydrolysis and produced acid from mannitol. It was negative for anaerobic growth (Tables 1, 2, 3).

Table 2: Biochemical and physiological characteristics of St7c5 strain

Characteristics	St7c5
Gram test	+
Motility in peptone water	-
Nitrate reductase	-
Kovac's oxidase	-
Gelatine hydrolysis	+
catalase	+
Caseine hydrolysis	+
H2S production	+
Urease production	-
Indole production	-
Growth at 4% NaCl	+
Growth on PH=4.5 PH>=6.5-8	 - +

-: negative reaction (no bacterial growth), +: positive reaction (bacterial growth)

The isolate that gave inhibitory activity was identified as belonging to the genus *Streptomyces*. It produced pigment on yeast malt agar (**ROTHROCK and GOTTLIEB,** 1981), and grows on medium containing 5% of NaCl.

The isolate of *M.pinodes* initiated growth when plated at 5cm from either *Streptomyces* streak. However, growth usually cease after 2-3 days.

Table 3 : Carbohydrate utilization by St7c5 strain

carbohydrates	St7c5
D-arabinose	+
glucose	+
D-fructose	+
Rhamnose	+
Raffinose	+
Lactose	-
Starch	+
Sucrose	-
Maltose	+
Manitol	+
Xylose	+

-: negative reaction (no bacterial growth), +: positive reaction (bacterial growth)

Unchallenged isolate of *M.pinodes* grew an average of 0.5-0.7 cm/day after a lag period, whereas in the presence of *Streptomyces* isolate, the average rate declined rapidly (fig1).

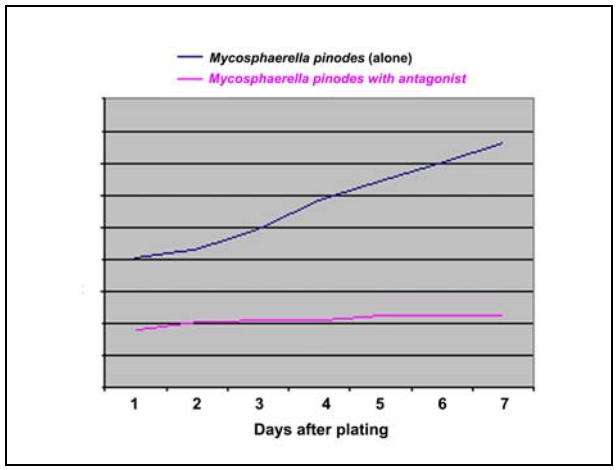

Figure 1: Average growth rate in axenic cultures and in the presence of Streptomyces antagonist

Greenhouse studies

Seed treatment with *Streptomyces* antagonist (St7c5) formulated in different organic and inert charges has given great increase in the percentage of seed emergence. Treatment with charge alone has also resulted in improvement the seed emergence compared with control. Moreover, the treatment with talc was substantially grater compared to the treatment with starch and pectin. Statistical analysis has revealed significant differences between the treatments compared to the controls. The most important emergence was observed with the combination *Streptomyces* – talc with 98.5 %.

Mycosphaerella foot rotting and blight symptoms:

The foot rot symptoms induced by *Mycosphaerella pinodes* on peas seedlings was reduced in treated plants compared to untreated seeds (30.5%). This was however less observed in talc formulation or with others charges (Table 4). The results were very significant between treatments compared to controls.

Symptoms were observed in plants issued from coated seed with either charges or biocontrol agent (5.2%).

Concerning the blight symptoms, the inoculated plants have presented necrosis on lower leaves and stem. The percentage of disease recorded approached 10%.

However, the development of disease was reduced when seed were formulated with either the charge or biocontrol agent. This disease varied from 4 to 7.5%. Furthermore, less than 1% of blight symptoms were observed on seedling issued from seed coated with association of charge and biocontrol agent.

DISCUSSION

Results obtained from the identification tests showed that actinomycetes isolates obtained that inhibit the fungus *Mycosphaerella pinodes* belong to the *Streptomyces*.

Many *Streptomyces* strains are known to produce antibiotics which are effective against bacteria, yeasts and fungi (El-ABYAK, *et al*, 1993).

Furthermore, the *Streptomyces* antagonist isolate could be used effectively to control the pea seed during and after the germination phase.

In fact, many pathogens are sensitive to *Streptomyces* antagonists (CHANG et KOMMEDAHLL, 1968; KOMMEDAHL, 1983; ROTHROCK and GOTTLIEB, 1981; WEINHOLD and BROWMANS, 1968, WALTER *et al*, 1995).

Activity of the isolate against *Mycosphaerella pinodes* suggests that the isolate can be effective for biological control agent. Test under greenhouse conditions showed that the biological agent enhances germination comparatively to the control.

In fact, this increase in germination has been noticed with other antagonist agent. Hence, WALKER et AL, (1998), suggested that the *Bacillus polymyxa* could enhance the germination. COOK, (1993), SABARTANAM et TRAQUAIR (2002), recently, observed that the formulation of seed with certain micro organism such as *Pseudomonas fluorescens*, and *Streptomyces* enhances considerably the germination.

Furthermore, the *Streptomyces* agent has also enhances the root and shoot growth. Such increases in plant growth may result from (i) control of deleterious root micro organisms , (ii) direct production of growth stimulating factors (i.e., hormones or growth factors) CRAWFORD, et AL. 1993), or (iii) increased nutrients uptake through enhanced root growth or promoted availability of necessary nutrients. Activity of biocontrol agents could also lessen concentrations of substances in soil that are inhibitory to plant growth (El-ABYAK *et al*, 1993).

In this context, MATHIVANAN *et al*, (2000b), THIRUP et AL, (2003), suggested that the use of certain biological control showed a positive influence in improving growth such as root length, dry weight, and plant height to 25% compared with the control.

Furthermore, the biocontrol agent reduces significantly the foot rotting symptoms that may be serious problems in seedlings when exposed to *Mycosphaerella pinodes* at early stages. In fact, many group of bacteria particularly fluorescent bacteria and *Streptomyces* have been reported to suppress the disease incidence different crops (WINDHAM *et al*, 1986; KLEPPER *et al*, 1988).

These bacteria of the genus *Streptomyces* control many soil disease by inhibiting the growth of fungal agent by production of antibiotics and sideriphores (MEHROTRA and SINGH, 1980, SAVITRY and GNANAMANI, 1987, BEALE, and PITT. 1990).

REFERENCES

[1]- BEALE, R. E., and PITT.D. 1990. Biological and integrated control of *Fusarium* basal rot of *Narcissus* using *Minimedusa polyspora* and other micro-organisms. Plant Pathology 39(3), 477 - 488

[2]- BOUZNAD.Z.1988. Contribution à la connaissance du genre Ascochyta chez les Légumineuses. Etude biologique, Ultrastructurale et Cytochimique des relations hôte parasite chez le couple *Ascochyta pisi lib* (*Pisum sativum L.*) thèse. Doc.Etat.123p.

[3]- BUCHANAN. R.D., and GIBONS. N.E.1974. Bergy's manual of determinative Bacteriology. 8thed. Williams & Wilkins, Baltimore.

[4]- CHANG I., KOMMEDAHL. T., 1968. Biological control of seedling blight of corn by coating kernels with antagonistics microorganisms. Phytopathology 58: 1395-1401.

[5]- COOK, R. J. 1993. Making Greater Use of Introduced Microorganisms for Biological Control of Plant Pathogens. Annual review of phytopathology 31, 53-80

[6]- CRAWFORD, D. L., LYNCH. J. M., WHIPPS, J. M., and OUSLEY. M. A. 1993. Isolation and Characterization of Actinomycete Antagonists of a Fungal Root Pathogen. Applied and Environmental Microbiology, 59(11): 3899-3905

[7]- El-ABYAK, M. S., El-SAYED, M. A, El-SHANSHOURY, A. R. and El-SABBAGH. S. M.1993. Toward the biological control of fungal and bacterial diseases of isolation and characterization of actinomycete antagonists of a fungal root their influence on plant growth. Plant Soil 56:475–483.

[8]- KLOEPPER. JW, LIFSHITZ.R, SCHROTH.MN.1988. Pseudomonas inoculants to benefit plant production. Annual Plant Sci 1: 60-64.

[**9]-** KOMMEDAHL. T., 1983. Biological control of soilborne pathogens: Introduction (abstract.).Phytopathology 73: 776.

[10]- MATHIVANAN. N, SRINIVASAN.K, CHELLIAH.S.2000a. Biological control of soilborne diseases in cotton, eggplant, okra and sunflower by Trichoderma viride.Z.Pflzkrank.PflShutz 107: 235-244.

[11]- MATHIVANAN.N, PRABAVATHY.V.R., VIJAYANANDRAJ.V.R.2005. Application of talc formulations of Pseudomonas fluorescens Migula and Trichoderma viride decrease the sheath blight disease and inhance the plant growth and yield in rice. J.Phytopathology 153:697-701.

[12]- MEHROTRA, R. S., and SINGH. P. J. 1980. Biological control of *Rhizoctonia bataticola* on gram by coating seed with *Bacillus* and *Streptomyces* spp. and pathogen. Appl. Environ. Microbiol. 59:3899–3905.

[13]- ROTHROCK. C.S., and GOTTLIEB, D.1981. Importance in antibiotic production in antagonism of selected Streptomyces species to two soilborne pathogens. J. Antibiot.34: 830-835.

[14]- SABARATNAM, S. TRAQUAIR, J.A.2002. Formulation of a *Streptomyces* biocontrol agent for the suppression of *Rhizoctonia* damping-off in tomato transplants. *Biological Control*. 23(3):245-253.

[15]- SAVITRY.S., GUANAMANICKAM.S.S.1987. Bacterization of peanut with Pseudomonas for biological control of Rhizoctonia solani and for enhanced yield. Plant Soil 102: 11-15.

[16]- THIRUP.L, JOHANSEN.A., WINDING.A.2003. Microbial succession in the rhizosphere of live and decomposing in barly roots as affected by the antagonistic strains Pseudomonas fluorescens D.R.54-BN14 or the fungicide IMIZALIL. FEMS Microbiol Ecol 43:383-392.

[17]- TIVOLI.B.1998. L'anthracnose de pois protéagineux, comprendre, évaluer sa nuisibilité pour mieux raisonner la protection de la culture. Phytoma 512 : 6-20.

[18]- WALKER, R, POWELL.A.A, SEDDON.B. (1998). Bacillus isolates from the spermosphere of peas and dwarf French beans with antifungal activity against Botrytis cinerea and Pythium species. Journal of Applied Microbiology 84 (5), 791–801.

[19]- WALTER M. YUAN AND. CRAWFORD. D. L.1995. Characterization of Streptomyces lydicus WYUC108 as a potential biocontrol agent against fungal roots and seed rots.Applied and Environnmental Microbiology, Vol. 61,(8): 3119–3128.

[20]- WEINHOLD, A.R., and BOWMAN, T. 1968. Selective inhibition of the potato scab pathogen by antagonistic bacteria and substrate of infoluence on antibiotic production. Soil Sci.28: 12-23.

[21]- WINDHAM, MT, ELAD. Y, BAKER.R.1986. A mechanism forincreased plant growth by Trichoderma spp. Phytopathology 76: 518-521.