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Cephalosporium maydis AS AFFECTED BY MAIZE ROOT EXUDATES AND ROLE OF THE FUNGAL METABOLITES IN PATHOGENESIS

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ABSTRACT

Cephalosporium maydis is a destructive microbial pathogen for the economic important crop maize in Egypt causing the late wilt disease. As pathogenesis is host and pathogen dependent, the effect of maize root exudates on *C. maydis* linear growth and the role of the fungal metabolites in the pathogenesis on maize were investigated. The obtained results revealed that root exudates of the resistant maize cultivar SC10 decreased the linear growth of *C. maydis* in Petri-dishes while those of the susceptible cultivar Balady had no effect. In addition to the known role of vessels plugging by the fungal biomass, responsibility of the fungal metabolites in pathogenesis was proved since the tested filtrates of *C. maydis* had deleterious effects on grain-germination, seedling growth and water conductivity in shoot parts. Injection of mature maize plants with *C. maydis* filtrates caused internal dark-brown to black discoloration.

Keywords: *Cephalosporium maydis*, fungal metabolites, maize, root exudates.

INTRODUCTION

The plant pathogenic fungus *Cephalosporium maydis* was recorded as the causal agent of the late wilt disease on the important crop maize in Egypt (Samra *et al*., 1963). Incidence and development of infection by *C. maydis* are host and pathogen dependent. Root exudates of maize plants are thought to affect the development of infection (Sayed-Ahmed, 1990). On the other hand, toxins produced by the wilting pathogens were proved to play a great role in the pathogenesis of such pathogenic fungal group (Gray & Chamberlain 1975; and Pandey *et al*., 1997).

The present study aimed to enlighten the effect of maize root exudates on *C. maydis* growth and the role of the fungal metabolites in the pathogenesis on maize.

MATERIALS AND METHODS

1- Isolation and identification of the pathogen:

Samples of maize plants showing typical symptoms of the late wilt disease were collected from some governorates of Egypt (Kafrelsheikh, El-Beheria, El-Dakahlya, El-Menia and Sohage) during the growing season 2001 and used to isolate the pathogenic agent using the method described by Awad (2002). The lower third to fifth internodes of diseased plants were thoroughly washed with running water and cut into small pieces of 1 cm in length. Pieces were surface sterilized by immersing in 0.5% sodium hypochlorite solution for 3 minutes, then, washed several times in sterilized water and blotted between two sterilized filter papers. Under aseptic conditions, internal tissues were transferred onto Petri dishes containing PDAY medium (Potato Dextrose Agar medium amended with 2 g yeast

extract/L as recommended by Abd El-Ghani, 1987). Dishes were incubated at 28 ± 1°C for 3-7 days and examined daily for occurrence of fungal growth. The growing isolates were examined microscopically and purified using the hyphal tip technique described by Dhingra & Sinclair (1995). Pure cultures of the obtained isolates were maintained on PDAY slants and kept at 4°C for further experiments.

Identification of the obtained isolates was carried out based on the morphological characteristics of growth and microscopic examination features. In addition, pathogenicity tests were performed according to El-Shafey *et al*. (1988) on different maize cultivars (SC.10, SC.123, TWC.310, Giza-2 and Balady). The percentages of diseased plants were recorded at 90 days after sowing. Fungal isolates graded as the highest and the lowest virulent and the plant cultivars graded as the resistant and susceptible were all selected for next experiments.

2- Effect of maize root exudates on *C. maydis* growth:

a) Preparation of root exudates:

To obtain the maize root-exudates, the procedure reported by El-Fangary (1975) was conducted. Large test tubes (2.5 X 20 cm) containing 5 cm depth of 5 mm diameter glass beads and 10 ml of Hogland & Snyders' 4-salt nutrient solution (prepared by mixing 1, 5, 5, and 2 ml molar solutions of KH_2PO_4, KNO_3, Ca $(NO_3)_2$ and $MgSO_4$, respectively and completed to 1 liter using tap water) were autoclaved and seeded with sodium hypochlorite surface-sterilized maize grains. Grains of the most susceptible (Balady) and the most tolerant (SC.10) maize cultivars were used. Ten replicates were prepared for each. Tubes were incubated at room temperature (28 ± 1◦C) under indirect light for 14 days. The nutrient solution was daily completed to be always kept at 10 ml. At the end of the period, the remaining nutrient solution were collected for each cultivar.

b) Effect of root exudates on *C. maydis* radial growth:

The root exudates solution of each maize cultivar was incorporated in PDAY medium at 1:1 ratio before distribution in 9 cm diameter Petri-dishes. Check dishes contained PDYA supplemented with the plan nutrient solution instead of the root exudates solution. Dishes were inoculated in the center with 5 mm culture discs of the fungus and incubated at 28±1ºC. Four dishes were prepared for each treatment. Linear growth, compared with the check treatment, was recorded after 6 days incubation period.

3- Effect of *C. maydis* metabolites on maize plant:

The culture filtrate of the most virulent isolate of *C. maydis* (isolate No.5) and the most susceptible maize cultivar (Balady) were used to perform the following testes. The isolate No.5 of *C. maydis* was grown for 30 days at 28ºC in 250 ml conical flasks, each containing 100 ml of PDY liquid medium. The liquid culture was filtrated through whatman No.1 filter paper and centerfugated at 6000 rpm for 20 minutes. After filtration through 0.4 µm membrane filter, the obtained filtrate was immediately used for treating of different maize plant organs as following:-

a) Effect on seed germination and seedling growth:

Grains of Balady maize cultivar were soaked in the isolate No.5 culture filtrate for 6 hrs. Grains soaked in sterilized PDY liquid medium were used as

check treatment. The treated grains were kept in Petri-dishes (10 grains/dish) on water moistened filter papers at 28ºC for a weak. Ten dishes were used for every treatment as replicates. Numbers of germinating grains were recorded when full germination took place in the check treatment. After germination, the seedlings were separated in test tubes containing sterilized tap water and allowed to grow forming shoot and root systems. After further 10 days, the length of tops and main roots of seedlings were measured

b) Effect on water conductivity:

To determine the effect of *C. maydis* culture filtrate on water conductivity, maize seedlings of 20 days-old were cut under water surface at the base of the stem and immediately placed in the filtrate poured in 10 ml. medical vials. A filtrate of un-inoculated medium and sterilized distilled water was used as check treatments. Three replicates were used for each test. The experimental plant materials were kept under the laboratory conditions and daily observed for wilting or toxicity symptoms. To measure the defect in water conductivity, the method of Wiese (1972) was performed. The red color Acid Fuchsine dye was dissolved in the above used aqueous phases to obtain solution of 0.5% Acid Fuchsine for each. Detached leaf blades of maize seedlings (20 days-old) of Balady cultivar were cut under water surface, and their cut ends were placed immediately in the prepared solutions. The blades were allowed to accumulate the dye for 4 hrs. Afterwise, the blades were harvested, cut into segments and dried overnight at 80-90ºC. Dye was extracted with water (50mg dry leaves /20 ml.) in a high-speed blender for 1 min. The ground mixture was filtrated through whatman No.2 filter paper and the absorption of the filtrate was measured at 542 nm (the absorption maximum for Acid Fuchsine) on spectrophotometer (MILTOROY, SPECTRONIC 1210). The uptake of Acid Fuchsine reflecting the conductivity of water was expressed as percentages compared with the check blades treated with Acid Fuchsine in water.

c) Effect on the stem internal tissues:

The sterilized culture filtrates, un-inoculated medium and distilled water were used for injection in maize plant stems. According to Ibrahim and Kamara (1972), 60 days-old field growing plants of maize were injected into the lower second internodes with 5 ml. using sterilized hypodermic syringes. Twenty plants were used for each treatment. After 30 days from injection, longitudinal sections were made and the internal reaction was observed.

RESULTS AND DISCUSSION

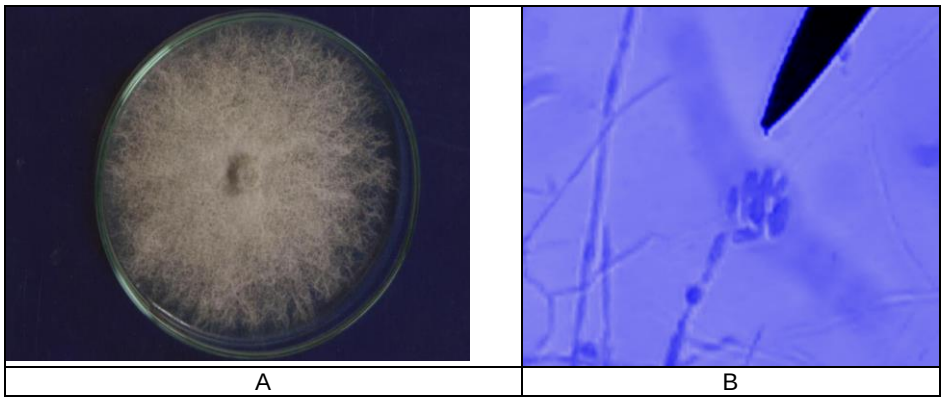
1- Isolation and identification of the pathogen:

As shown in Table (1) and Fig (1), the main common cultural and microscopic characters coincided with that of the fungus *Cephalosporium maydis*, Samra, Sabet and Hingorani recorded by Samra *et al*. (1963).

The pathogenicity tests revealed that the obtained isolates differed in their virulence. The degree of disease incidence varied depending on the isolate and the tested cultivar. However, all the tested isolates proved their pathogenic character of *C. maydis* since they infected and diseased one or more of the tested cultivars causing typical symptoms of the late wilt disease (Fig.2).

Table (1): Cultural and microscopic characters of *C. maydis* isolates.

Characters	Description
Colony color	Felty, white at first, but turning gray by aging
Colony margin	Rhizoid appearance in a clock wise direction
Mycelium growth	Moderately rapid, covering the agar surface of a 9- cm Petri-dish in 6-days.
Hyphae	Hyaline, septate and branched
Conidiophores	Straight, 400 µ in length
Conidia	Produced exogenously at apices of the conidiophores and several spores aggregated in heads, hyaline, straight, single celled, oblong and measured 3.6-14 X 3-3.6 µ.(7.2X3.5 µ)
Sclerotia	Small sclerotia-like bodies consisting of a few thick walled, dark colored cells ,might appear in old cultures.



A B

Fig (1) Morphology (A) and microscopic feature (B) of *C. maydis* growth.

The most virulent isolate of *C. maydis* was the isolate No.5 which was isolated from Sohage governorate. It caused 25.75% as mean of disease incidence on the tested maize cultivars. Furthermore, it attacked and diseased 4 of the tested cultivars, i.e. SC.123, TWC.310, Giza.2 and Balady where the recorded disease incidence percentages were 10%, 7.14%, 42.38% and 69.23%, respectively.

The highest recorded disease incidence percentage (69.23%) was obtained on the Balady cultivar. In contrast, the maize cultivar SC.10 showed high resistance to the infection by all the tested *C. maydis* isolates, since no symptoms of the disease were recorded in the presence of any isolate.

Fig (2): Close up on the stem bases suffering from the late wilt disease.

2- Effect of maize root exudates on *C. maydis* radial growth:

As shown in Fig. (3) and Tab. (2), the linear growth of *C. maydis* was significantly inhibited by root exudates of the resistant plants. The diameter of *C. maydis* growth did not exceed 6 cm. On the other hand, the diameter growth of *C. maydis* in presence of the susceptible plants root exudates (8.5 cm) did not significantly differ from the diameter growth of *C. maydis* on the blank substrate (8.966 cm).

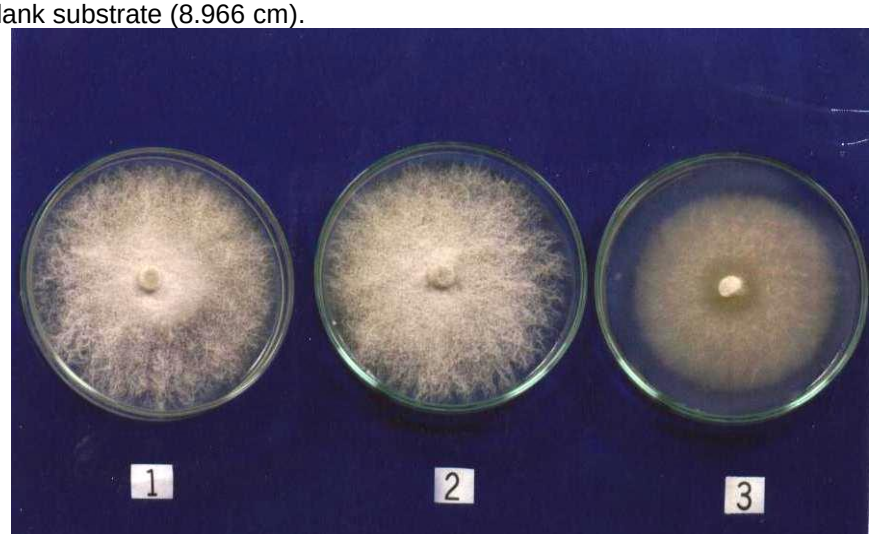


Fig (3): Effect of root exudates of Balady (2) and SC.10 (3) maize cultivars on linear growth of the most virulent isolate No.5 of *C. maydis* comparing with the control treatments (1).

Table (2): Effect of root exudates on linear growth of C*. maydis* after 6 days inoculation.

Treatments (Growth on medium containing)	Linear growth* (cm)	% Reduction **
Root exudates of SC.10	6.000 a	33.056
Root exudates of Balady	8.466 b	5.566
Control	8.966 b	-

*** In the same column, values having the same letter are not significantly different at P = 0.05.**

**** Compared with control treatment.**

Although El-Laithy (1996) found amounts of sugars and free and conjugated phenols in root exudates of the resistant cultivar (Giza-2) higher than in the susceptible cultivar (Balady). Moreover, Park *et al.* (2004) isolated yellow and colorless antifungal factors from maize root exudates which inhibited the growth of the soil-borne plant pathogen *Fusarium oxysporium f. sp. melongenae*. The chemical structure of the maize root exudates, antagonistic to C. maydis, has not been yet identified. Such inhibitor factors are needed to be chemically identified.

3- Effects of *C. maydis* metabolites on maize plant:

Effects of culture filtrate of the most virulent isolate (isolate No.5) on germination, seedling growth, water conductivity and stem internal tissues of the most susceptible maize cultivar (Balady) were studied.

a) Effect on seed germination and seedling growth:

Soaking of maize grains in the fungal filtrate for 6 hrs before seeding in Petri-dishes inhibited their germination to 23.33% as compared with those soaked in blank water (Fig. 4 and Tab. 3).

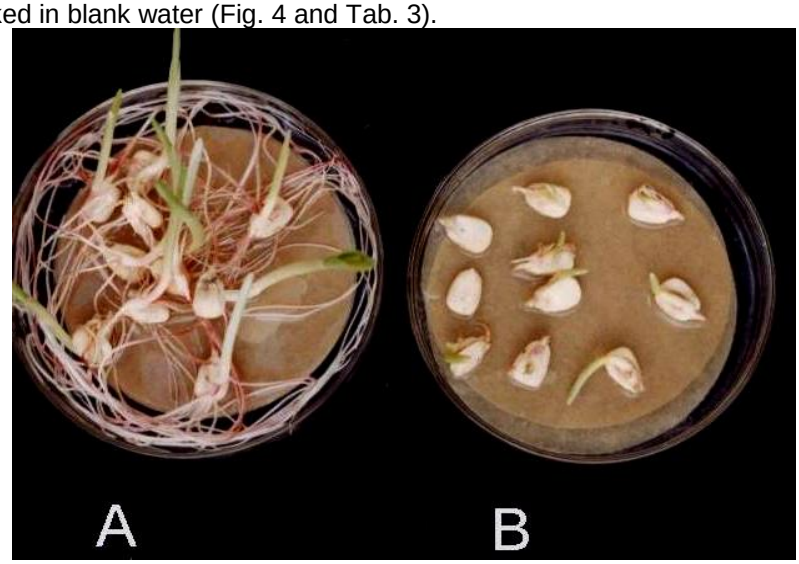


Fig (4): Effect of *C. maydis* culture filtrate on seed germination of Balady maize cultivar (A = control, B = culture filtrate of the most virulent isolate No. 5).

Fig (5): Effect of *C. maydis* culture filtrate on shoot and root growth of Balady maize cultivar (A= water, B = Culture filtrate).

Table (3): Effect of culture filtrate of *C. maydis* on grain germination and seedling growth of Balady maize cultivar.

Treatments	% germination	Shoot length (cm)	Root length (cm)
Water	100	8.5	9.0
Culture filtrate	23.33	4.5	0.8

The seedlings raised from the above mentioned treated grains suffered from decrease in their shoot and root growth (Fig. 5 and Table 3). Lower shoot and root lengths (4.5 cm and 0.8 cm, respectively) were recorded, while these lengths reached 8.5 cm and 9cm, respectively in the check treatment.

b) Effect on water conductivity:

Figure (6) shows that immersion of the shoot system (stem bearing leaves) in culture filtrate of *C. maydis* (isolate No.5) resulted in phytotoxic symptoms leading to the wilt (Fig. 6: C). In contrast, the check treatments (using blank water or the liquid medium) had no phytotoxic features (Fig 6: A and B, respectively). In the presence of *C. maydis* culture filtrate, the estimated water conductivity decreased to be 26.76% as compared with the blank water treatment (Table 4).

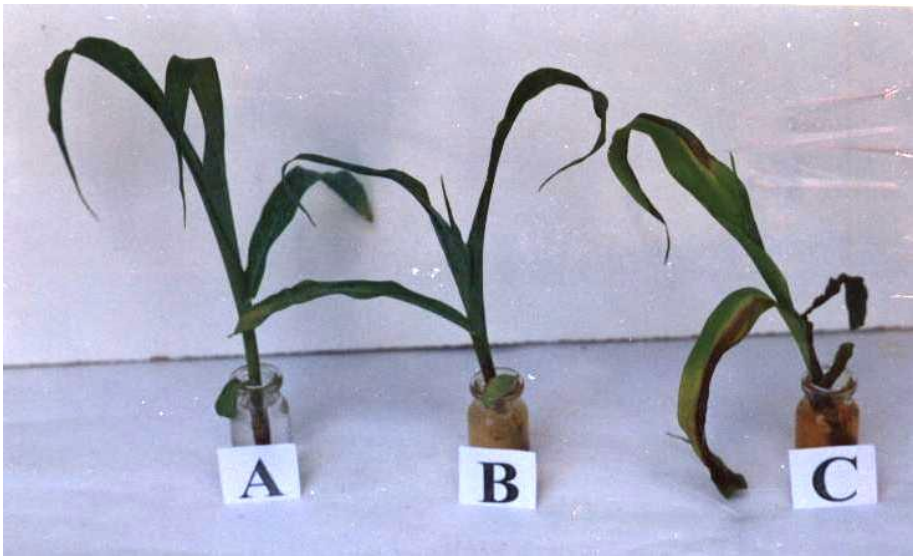


Fig (6): Effect of culture filtrate of the most virulent isolate (No.5) on Balady maize cultivar seedlings after 6 days of base immersion (A = water, B = liquid medium, C = culture filtrate.

Table (4): Estimated water conductivity in detached maize plant leaves as affected by the culture filtrate of *C. maydis* (isolate No. 5).

Treatment (Immersion in)	Water conductivity %*
Culture filtrate	26 .76
Liquid medium	64.39
Water	100

*** Estimated from the absorption values of the Fuchsin acid dye spectrophotometrically measured at 542 nm.**

c) Effect on the stem internal tissues:

Injection of mature maize plants with the *C. maydis* (isolate No5) culture filtrate caused internal dark-brown to black discoloration (Fig. 7: B). This result can explain that obtained by Moursy (1978) where inoculation of maize stems with *C. maydis* itself (mycelium and spores) caused internal brown discoloration though the symptoms of the late wilt disease could not be developed.

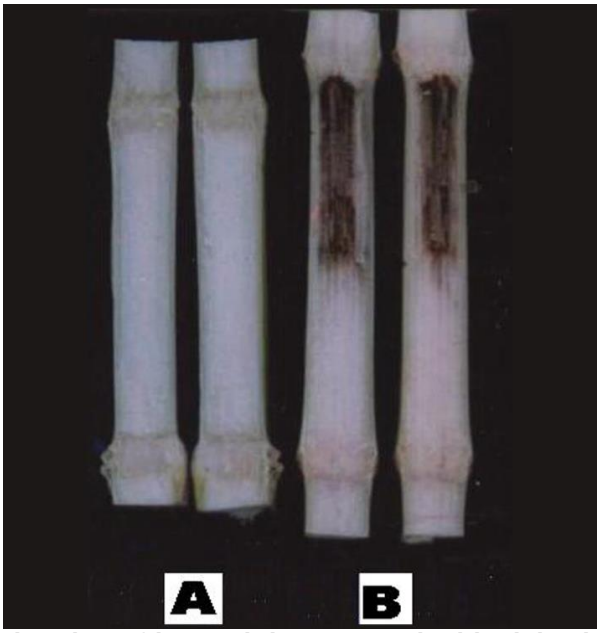


Fig.(7): Discoloration of internal tissues resulted by injection of culture filtrate of the most virulent isolate No. 5 (A= water, B= *C. maydis* culture filtrate).

In the present study, the isolate No.5 of *C. maydis* which was isolated from Sohage governorate showed the most virulence. Its propagules could be observed in prepared cross sections of infected plants colonizing the xylem vessels (data not shown). Although El-Shafey et al. (1988) and Khalifa (2000) stated that *C. maydis* is a true vascular fungus and vessels occlusion may be the most important factor preventing the water conductivity. The present study proved that the fungal metabolites are implicating in this respect. It was proved that the virulent isolate No.5 of *C. maydis* can excrete chemical factor(s) toxic to the host plant. The excreted toxic metabolites inhibited the grain germination, the shoot and root growth, and water conductivity in the detached leaves of maize, and when injected into stems of the mature maize plants, the internal tissues turned to dark-brown and black discolored tissues. In an earlier study carried out by Sadik (1973), although culture filtrates of *C. maydis* had no effect on germination percentage of maize grains, length and weight of roots and tops were significantly inhibited and color of roots changed to brown. However, studies for chemical identification of the pathogen *C. maydis* metabolites having toxicity on the host maize plants are needed.

The present study offered simple tests valuable to save time and efforts spent in screening programs for evaluating maize germplasms as additional sources of resistance through testing their root exudates against the pathogenic *C. maydis* isolates. In addition, the aggressiveness of *C. maydis* isolates could be simply verified through testing their deleterious effects on both grain-germination and seedling-growth of maize.

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تـأثر الفطـر *Cephalosporium maydis* بـإفرازات جـذور نبـات الـذرة الشامية ودور الإفرازات الفطرية في حدوث المرضية

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يعتبر الفطر *Cephalosporium maydis* Samra, Sabet and Hingorani من الميكروبات شديدة الضرر بمحصول الذرة الشامية ذات الأهمية الاقتصادية في مصر مسبباً له مرض الذبول المتأخر. وحيث أن المرض يعتمد في حدوثه على خصائص كل من العائل والمسبب فقد تناولت هذه الدراسة تأثير إفرازات جذور الذرة على نمو الفطر *C. maydis*، وكذلك دور إفرازات هذا الفطر في إحداث المرضية. وقد أوضحت نتائج هذه الدراسة أن:-

1- أدت إفرازات الجذور لصنف الذرة المقاوم للعدوى(هجين فردي 10) إلى تقليل النمو القطري للفطر في أطباق بتري بينما لم يتأثر نمو الفطر بإفرازات جذور الصنف القابل للإصابة (بلدي).
2- كان لراشح المزرعة السائلة للفطر *C. maydis* تأثيراً ضاراً على إنبات حبوب الذرة ونمو بادراته، وكذلك على عملية سريان الماء بالمجموع الخضري للبادرات، وبحقن هذا الراشح في السيقان البالغة لوحظ تلون الأنسجة الداخلية باللون البني الداكن أو الأسود. وبذلك فإن هذه النتائج تثبت مسؤولية إفرازات الفطر *C. maydis* عن إحداث الضرر بعائلة بالإضافة لما هو معروف مسبقا عن الضرر الناتج من انسداد أوعية النبات العائل بنموات هذا الفطر.

بذلك تساعد هذه الاختبارات البسيطة في توفير الوقت والجهد المبذول في برامج انتخاب النباتات ذات التراكيب الوراثية الخاصة بالمقاومة وذلك باختبار قدرتها على تكوين إفرازات جذرية مثبطة للمسبب الميكروبي للمرض، وكذلك في تقدير درجة شراسة العزلات الخاصة بالمسبب الميكروبي للمرض باختبار قدرتها على إفراز مواسد لها دور في المرضية.