EFFECT OF 2,4-DIACETYLPHLOXOGLUCINOL PRODUCED BY *Pseudomonas fluorescens* Q2-87 ON β-1,3 GLUCANASE AND CELLULASE OF *Pythium ultimum*

Youssef, S. A.; K. A. H. Tartoura; M. Nair and J. M. Vargas

1- Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.
2- Horticulture Department, Michigan State University, E. Lansing, MI 48824.
3- Plant Pathology Department, Michigan State University, E. Lansing, MI 48824

ABSTRACT

In our previous study we demonstrated that the biocontrol agent, *Pseudomonas fluorescens* Q2-87 producing the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG), has the ability to antagonize the phytopathogenic *Pythium* spp. by degradation of hyphal cell walls and inhibition of growth. Thin-layer chromatography (TLC) as well as high performance liquid chromatography (HPLC) techniques were used to confirm the production of 2,4-DAPG from the biocontrol agent *P. fluorescens* Q2-87. Activities of polysaccharide degrading enzymes, β-1,3 and β-1,4 glucanase (cellulase), and their relation to *Pythium ultimum* Frow cell wall degradation in relation to exogenous application of 2,4-DAPG were investigated during the first 72 h after treatment. Higher levels of β-glucanases in treated *P. ultimum* comparing to that in control were detected. Glucanase activity reached its highest peak at 12 h; however, cellulase activity reached its highest peak at 24 h after application. Native polyacrylamide gel electrophoresis also confirmed the activities of such hydrolytic enzyme in treated *Pythium mycelia*. Conclusion that bacterial cell-wall degrading enzymes origin are excluded since 2,4-DAPG isolation procedure resulted in 2,4-DAPG-free from such enzymes. The present results suggest that exogenous application of 2,4-DAPG to *Pythium* resulted in higher levels of such cell-wall degrading enzymes that participated in hyphal degradation of the oomycete *Pythium*.

**Keywords:** 2,4-Diacetylphloroglucinol, β-glucanase activities, *Pseudomonas fluorescens* Q2-87, *Pythium ultimum*

INTRODUCTION

*Pseudomonas fluorescens* has been used as a biocontrol agent against various plant pathogens because of its capability to produce one or more antibiotics (Hill et al. 1994; Cornin et al. 1997; Duffy and Défago 1989; Thomashow et al. 2000; Landa et al. 2002). Production of 2,4-DAPG is one of the antibiotics produced by several strains of *P. fluorescens*, including Q2-87 (Shanahan et al. 1992; Keel et al. 1992; Fenton et al. 1992; Harrison et al. 1993; Bonsall et al. 1997; Dunne et al. 1998). *P. fluorescens* Q2-87 was isolated from the rhizosphere of wheat grown in a take-all-suppressive soil and inhibited *Gaeummannomyces graminis var. tritici* in vivo and in vitro by producing the antibiotic 2,4-DAPG (Harrison et al. 1993). Q2-87 strain of *P. fluorescens* producing 2,4-DAPG has the ability to antagonize the phytopathogenic *Pythium* spp. by degradation of hyphal cell wall and inhibition of mycelial growth (Youssef et al. 2003). Since β-1,3 glucan and cellulose (β-1,4 glucan) are two of the main structural components of
oomycete plant pathogens (Barthnicki-Garcia and Wang 1983) and cell wall-degrading enzyme activities have been described among fungi, algae and bacteria (Pitson et al. 1993), \( \beta -1,3 \) glucanase and cellulase appear to be involved in the antagonistic interaction process of Pythium spp. by P. fluorescens Q2-87. The physiological role of 2,4-DAPG produced by this strain in this process against P. ultimum in relation to cell wall-degrading enzymes is not studied yet.

The present investigation was carried out to achieve the following goals: (a) confirmation that whether or not Q2-87 produces the antibiotic 2,4-DAPG under the experimental condition, if so, (b) determination of \( \beta -1,3 \) and \( \beta -1,4 \) glucanase levels in Pythium cells, and (c) determination of the \( \beta - \)glucanase source that participated in hyphal cell wall degradation.

**MATERIALS AND METHODS**

**Organisms and culture conditions**

Lyophilized P. fluorescens strain Q2-87 (NRRL B-23374) was obtained from the Agricultural Research Service Patent Culture Collection (National Center for Agricultural Research, USDA, Peoria, Illinois). Cultures were grown in 100 ml tryptone soy broth (TSB, 30 g/ L, Difco Lab, Detroit, MI) for 24 h. Cultures were maintained at 28°C in tryptone glucose yeast medium (TYG) containing 5 g tryptone, 5 g yeast extract, 1.0 g glucose, 1.0 g KH₂PO₄ and 15 g agar in 1 liter-distilled water. The pH value was adjusted to pH 6.5. P. ultimum was isolated from cowpea in Georgia and kindly provided by Dr. Jeff Hoy (Louisiana State University). Pythium cultures were grown in solid V8 medium which consisted of 200 ml V8 juice, 3.0 g CaCO₃, 15.0 g agar, 820 ml distilled water. Following experiments were conducted in potato dextrose broth (PDB, 24 g/L, Difco Lab) and potato dextrose agar (PDA, 39 g/L).

**Confirmation that 2,4-DAPG produced by P. fluorescens**

Natural product 2,4-DAPG was isolated according to a modified method of Shanahan et al. (1992). Strain Q2-87 was grown in PDB for five days in a rotary shaker (250 rpm) at 24°C. Bacterial cells were harvested after centrifugation for 10 min at 6000g and the supernatants were filtered through 0.45 and 0.22 \( \mu \)m nitrocellulose filter. One liter of filtrate was lyophilized and yielded 15.0 g powder. The resultant freeze-dried powder was homogenized in 90% aqueous methanol (MeOH) (ratio 1: 4, w/v) followed by centrifugation at 15000g for 30 min. The supernatant was decanted and evaporated in vacuo at 30°C using rotary evaporator and the remaining pellets was dried, kept at 4°C and labeled fraction A. The aqueous extract (8.0 g) was redissolved in MeOH (10 ml) and precipitated by chloroform (CHCl₃) to remove sugars. The precipitate was filtered off, dried under vacuum and labeled fraction B. The filtrate MeOH/CHCl₃ was evaporated in vacuo to dryness and yielded 2.5 g which labeled fraction C. Bioassay preliminary experiments indicated that fraction C only exhibited an antagonistic activity against P. ultimum by in vitro inhibition assay described below. Therefore, an appropriate sample of this fraction was redissolved in 95% MeOH; 5-10 \( \mu \)l was chromatographed as well as authentic 2,4-DAPG.
on thin-layer chromatography (TLC) plates (Silica gel 60 F_{254}, Merck) with an chloroform: methanol (12:1 v/v) as mobile phase. The plates were viewed under UV at 254 and 360 nm and the UV visible spots were marked and further confirmed by spraying with 1% (w/v) vanillin in sulfuric acid (95%). Rf values were calculated for all bands. These bands were marked under UV light, individually scrapped from TLC plates, eluted with MeOH and subjected to in vitro inhibition assay.

For analytical high performance liquid chromatography (HPLC), 20 μl aliquots of MeOH extracts having antagonistic activity was analyzed by reversed-phase HPLC (0.8 x 10 cm Waters Novapak radial compression cartridge) eluted with a 0.1% trifluoroacetic acid-acetonitrile linear gradient (0 to 100% over 25 min, 100% for 5 min). The retention time of 2,4-DAPG was 14.5. Identification of 2,4-DAPG was confirmed by adding authentic 2,4-DAPG to a part of the sample.

In vitro antagonistic activity bioassay

Preliminary experiment indicated that the only band containing antifungal activity coincided with the UV fluorescent band at Rf 0.82. Consequently, in vitro antagonistic activities of the synthetic 2,4-DAPG (purchased from Toronto Research Chemicals Inc., Toronto, Canada) and the natural product produced by Q2-87 strain were assayed on PDA media according to a modified method of Vincent et al. (1991). Based on preliminary experiments, media was supplemented with either fraction C containing 2,4-DAPG at concentration 0.25 g/100 ml, or 5-7 μg/ml synthetic 2,4-DAPG. 5 mm mycelial plugs from the actively P. ultimum culture grown at 24°C for 3 days were placed in the center of the Petri dishes containing 20 ml of the amended medium. Bacterial strain Q2-87 was streaked in the middle of other Petri dishes containing PDA media as a positive control. After 24 h of pre-incubation at 24°C, mycelial plugs were placed 27 mm distant from each side of the bacterial streak. Inhibition zones were obviously seen after 48 h. Six replicates per treatment were performed.

Natural 2,4-DAPG treatment and sampling

Pythium mycelia were grown in freshly prepared PDB media for 4 days at 24°C in a rotary shaker, then the media replaced by other fresh ones supplemented with fraction C containing 2,4-DAPG (2.5 g/L, w/v) under sterilized conditions and 2,4-DAPG-free media was used for the control. Incubation condition was identical to those for Pythium growth. Starvation conditions did not occur throughout the experiment because some PDA was transferred intracellular of the mycelium, thus giving growth support during the time-course study. Treated and untreated Pythium mycelia were collected at specified times and quickly frozen in liquid nitrogen. Samples were weighed, frozen immediately in liquid nitrogen, lyophilized followed by re-weighting and used for enzymatic assay. Levels of β-glucanases, their specific activities, and total soluble protein were without significant variations during the time-course study. Consequently, control corresponded to zero-time.

Protein extraction

Dried mycelia was ground with a mortar and pestle under liquid nitrogen. The ground matter was homogenized (2 h, 4°C) in 0.05 M
phosphate buffer (1/3 w/v, pH 5.0). The homogenate was centrifuged twice at 10,000g at 4°C, the supernatant was collected and Protein concentration was determined according to Bradford (1976) using protein assay dye reagent (Bio-Rad Laboratories, Richmond, CA). Aliquots of each sample were precipitated in acetone (1/2, v/v, pH 4.5); acetone traces were vacuum dried, and the powder was resuspended in 0.05 M sodium acetate buffer (pH 5.0) and kept at -20°C. This fraction was immediately used for the determination of β-1,3 glucanase and cellulase activities.

**Determination of enzymatic activities**

β-1,3-glanucanase (EC 3.2.1.6) activity was assayed by a modification of the method of Lima et al., (1997) using Azurine-crosslinked pachyman (AZCL-pachyman; Megazyme) as the substrate. The complete reaction mixture (0.5 ml, 37°C) contained 225 μl crude enzyme preparation extracted with acetone, 25 μl AZCL-pachyman (10 mg ml⁻¹) and 50 mM sodium acetate buffer (pH 5.0). The mixtures were incubated for 20 min with moderate shaking. The reaction was stopped by adding 700 μl of Tris (20% w/v), incubated at room temperature for 3-4 min and then briefly centrifuged and the amount of blue soluble dyed fragments released from AZCL-pachyman was determined spectrophotometrically at 595 nm. Quantification of enzyme units was determined using a calibration curve constructed by plotting the amount of glucanase units and their optical densities and performing a linear regression. The method was linear (R>0.99) in the analyzed amount range of 0.005 to 0.25 units of glucanase. The activities were expressed as units h⁻¹ g⁻¹ dry weight (DW).

β-1,4-glucanase (EC 3.2.1.4) activity was assayed by a modified method of Worthington (1988) using cellulose (Sigmacell type 20, Sigma) as a substrate. The complete reaction mixture (0.5 ml, 37°C) contained 250 μl crude enzyme preparation extracted with acetone, and 250 μl 1% cellulose dissolved in 50 mM sodium acetate buffer (pH 5.0). The reaction mixtures were incubated at 37°C for 120 min followed by transferring into an ice bath for 10 min, then, centrifuged for 5 min at 2°C to clarify the supernatant. Into a cuvette, 3 ml glucose (hexokinase) determination (Sigma Stock No.16-10) was added and equilibrated to 25°C, then 0.1 ml of the supernatant was then supplemented. Immediately mix by inversion and record the maximal increase in absorbance for 5 min at 340 nm. Units ml⁻¹ enzyme = (ΔA340 test- ΔA340 blank)(3.1)(5)(df)

\[
(6.22)(2)(1)(0.1)
\]

Where 3.1 = final volume (in ml) of step 2, 5 = total volume (in ml) of reaction mix (step 1), df = dilution factor, 6.22 = millimolar extinction coefficient of β-NADH at 340 nm, 2 = conversion factor for 2 h to 1 h, 1 = volume (in ml) of cellulase used in step 1, 0.1 = volume (in ml) from step 1 used in step 2. The activities were expressed as units h⁻¹ g⁻¹ FW. All data are the means of three independent samples.

**Detection of β-1,3 glucanase and β-1,4 glucanase isoforms by native PAGE**

Non-denaturing PAGE [12% (w/v) acrylamide] was carried out without SDS using the buffer system according to Laemmli (1970). Detection of β-1,3 glucanase and cellulase activities during maximum activities measured.
spectrophotometrically, 12 and 24 h, after application, respectively, was performed by equilibrating in 50 mM potassium acetate buffer, pH 5.0, and then incubated in 25 mM potassium acetate buffer amended with 0.5% laminarin (Sigma), pH 5.0 for 45 min at 37°C. After incubation, the gel was washed with distilled water several times and incubated with 1 M NaOH amended with 0.15% 2,3,5-triphenyltetrazolium chloride in boiling water for 5 min. Red bands indicate β-1,3glucanase activity (Kim and Hwang 1997). In relation to cellulase activity, the gel was equilibrated by immersion in 0.25 M Tris-maleate buffer pH 5.0 for 10 min and blotted for 3.0 h at 37°C against substrate-containing buffered agar (0.5%, w/v) carboxymethylcellulose, CMC (Sigma, C-4888), 1.5% noble agar (Difco) in 0.1 M Tris-maleate buffer pH 5.0, as described by Bertheau et al. (1984). The agar gel was gently soaked for 0.5 h in 1% (w/v) Congo red, rinsed in water and 1 M NaCl, then immersed and soaked in 1 M NaCl for 20 min. After repetition of this coloring protocol, the gel was incubated in 1 M NaCl overnight. White bands indicate cellulase activity on a red background. Protein concentration was determined according to the method of Bradford (1976) using Bio-Rad protein assay. Bovine Serum Albumin (BSA) was used as the substrate. β-1,3 glucanase and cellulase activities were repeated three times with identical results.

**Determination of the source of β-glucanases**

Strain Q2-87 was grown in PDB supplemented with physiological concentrations of β-glucanases inducers, i.e. laminarin and CMC, for five days in a rotary shaker (250 rpm) at 24°C. Bacterial cells were harvested and the supernatants were collected as described above. This supernatant was subjected for assaying β-glucanases at the same manner as previously described.

**RESULTS**

**Confirmation that 2,4-DAPG is produced by P. fluorescens Q2-87**

A synthetic sample of 2,4-DAPG comigrated with fraction C on TLC and these compounds formed a brilliant yellow derivative when TLC plates were visualized with diazosulfanilic acid. TLC plates developed with fraction C showed presence of three bands as detected under UV radiation at 254 and 365 nm and further visualized after spraying with diazosulfanilic acid (Fig.1). An orange-yellow spot at ]]=0.82 corresponded to the presence of the authentic 2,4-DAPG was observed. One of these bands containing antifungal activity that was coincided with the UV fluorescent band at ]]=0.82. When this band was marked under UV light, scraped from TLC plates, eluted with MeOH, then filtered through a 0.2 μm pore-sized filter. The filtrates were concentrated, reinjected onto the HPLC and eluted as a single peak with a retention time of 14 min (Fig. 2). Furthermore, analysis by HPLC revealed that the synthetic and natural 2,4-DAPG exhibited identical retention times. Thus, in addition to TLC, HPLC also provides a mean to identification of the DAPG produced by P. fluorescens Q2-87.
Fig. 1: TLC plate shows authentic 2,4-DAPG band (A) and fraction C bands (B). Fraction C shows a spot corresponded to the presence of the authentic 2,4-DAPG.
Fig. 2: HPLC chromatographic profiles of 2,4-DAPG isolated from P. fluorescens Q2-87. Arrow in (A) represents natural 2,4-DAPG; arrow in (B) represents natural 2,4-DAPG after adding authentic 2,4-DAPG.
Youssef, S. A. et al.

Pythium inhibition bioassay

The biological activities of fraction C, authentic 2,4-DAPG, and the bacteria showed inhibitory effectiveness on Pythium growth (Fig. 3). Fraction C at 0.25 g/100 ml and authentic 2,4-DAPG at 50 μg/ml completely inhibited Pythium growth in vitro. Plates were streaked with P. fluorescens Q2-87 and inhibited mycelial growth.

Effect of natural 2,4-DAPG on β-1,3 glucanase activity

Higher levels of β-1,3 glucanase and its specific activity were found in treated Pythium relative to the untreated one during the time-course of study reaching the maximum level during 12 h after application (Fig. 4). Between 12 and 24 h, these activities gradually decreased. Total soluble protein level was also found to be higher in treated sample comparing to the control during the time-course of study reaching the maximum level during 8 h followed by a gradual decrease.

Effect of natural 2,4-DAPG on cellulase activity

As a response of natural 2,4-DAPG treated mycelium, cellulase activity and its specific activity levels were increased during the time-course of study and reaching the maximum levels during 24 h compared to control (Fig. 5). Total soluble proteins also increased during most of the time-course of study comparing to control and reached the maximum level during 6 h after application. Thereafter, it was gradually decreased.

Detection of β-1,3- and β-1,4- glucanase by native PAGE

Higher β-1,3- and β-1,4- glucanase isozymes were detected in 2,4-DAPG treated samples compared to untreated ones as shown in Fig. 6.

Source determination of β-1,3- and β-1,4- glucanase

β-1,3- and β-1,4- glucanase activities were not detected by means of either spectrophotometric assay or native electrophoretic technique in the 5 day-old culture supernatants of the Q2-87 strain which was either supplemented with β-1,3- and β-1,4- glucanase inducer or not. However, such enzymatic activities were present in Pythium mycelium treated with 2,4-DAPG (Figs. 4 and 5)

DISCUSSION

Production of antibiotics is an important mechanism proposed for the antagonistic activity of P. fluorescens against soil-borne plant pathogens (Shanahan et al. 1992; Howie and Suslow 1991). 2,4-DAPG has been shown to be the main antifungal compound produced by P. fluorescens strain Q2-87 (Bonsall et al. 1997). In the present work, production of the secondary metabolite 2,4-DAPG was confirmed by means of TLC and HPLC under the experimental conditions used in this investigation (Figs. 1 and 2). The present results are in agreement with Harrison et al. (1993) who reported that Q2-87 produces both mono- and diacetylpheoroglucinol, 2,4-DAPG is only active in exhibiting antagonistic activity against Pythium. However, the authors did not identify monoacetylphloretoglucinol and its inhibitory effectiveness in fraction C.
Fig. 3. Light micrographs of \textit{in vitro} inhibition assay of \textit{P. ultimum} on PDA. A, C, and F. Control; B, D, and F represent inhibition resulted from \textit{P. fluorescens} Q2-87, fraction C, and synthetic 2,4-DAPG, respectively. In D and F, the \textit{Pythium} did not grow.
Fig. 4: Effect of 2,4-DAPG produced by *P. fluorescens* Q2-87 on glucanase activity, specific glucanase activity, and total soluble protein of *P. ultimum* during the time-course indicated. Time-zero sample represents control.

In a previous study, degradation of *Pythium* cell walls occurred after treatment with the biocontrol *P. fluorescens* Q2-87. The source of the cell-wall polysaccharide degrading enzymes that caused degradation of cell wall was unknown (Youssef *et al.* 2003). Since *Pythium* cell wall consists mainly of β-1,3- and β-1,4-linked glucans (Bartnicki-Garcia and Lippman, 1973); it requires glucanase and cellulase for lysis (Bartnicki-Garcia and Wang, 1983).

In the present work, activities of cell wall-polysaccharide-degrading enzymes, β-1, 3- and β-1,4-glucanase, showed higher activities in *Pythium* cells after application with 2,4-DAPG. The activities of both enzymes, their specific activities and total soluble proteins exceeded the control by 2-5 times during most of the time-course study as compared to control (Figs. 4, 5, and 6). The present data also indicated that untreated healthy *Pythium* having a low level of β-glucanases production. Of course, presence of these enzymes in oomycete is essential to hyphal tip growth, morphogenesis and for changes in the cell wall composition during morphogenesis, as also suggested by Bartnicki-Garcia and Wang (1983), and Pitson *et al.* (1993).
Fig. 5: Effect of 2,4-DAPG produced by P. fluorescens on cellulase activity, specific cellulase activity, and total soluble protein of P. ultimum during the time-course indicated. Time-zero sample represents control.

The loss of the *Pythium* ability to control the expression of these enzymes following 2,4-DAPG application is probably caused by disruption of the regulatory mechanism; at least, because of the plasma membranes have been reported to be a potential target for antibiotics (Lewis and Papavizas 1987). It seems likely that the production of low levels of hydrolytic enzymes, e.g. β-1,3 glucanases, may have allowed the release of elicitor-active molecules in the presence of 2,4-DAPG, e.g. β-1,3 glucans, from *Pythium* cell walls, which further induce synthesis of these enzymes. Alternatively, 2,4-DAPG produced by Q2-87 could be a signal molecule. Alteration of the lipid composition of the plasma membrane of *Pythium* hyphae, possibly resulting from the binding of such molecules, may have induced deregulation of membrane-bound enzymes, resulting in increasing cell wall degrading enzymes. Although significant increase of β-1,3 glucanase and cellulase activities have been observed in mycelial hyphae of *Pythium* after application, it has not been elucidated if the increased activities were due to changed enzymatic properties, increased translation, or increased gene expression. If the induction of such enzymes was found to be due to an increase in the corresponding m-RNA level, increased translation and/or increased gene expression it might explain the response of the *Pythium* cells to 2,4-DAPG.
Thus, the latter cases would be confirmed. Additionally, Figs. 4 and 5 show that cellulase activity was peaked later compared to β-1,3 glucanase activity. This may be explained by the fact that cellulotic β-1,4 glucans are embedded in a matrix of amorphous material and successful cell-wall degradation may depend on the activity of more than one enzyme. As a result, β-1,4 glucan seems to be protected by noncellulosic β-1,3 glucan, and is not ready accessible to β-1,4 glucanase as also reported by Bartnicki-Garcia and Wang (1983). Thus, it is likely that β-1,4 glucanase activity is preceded by, or coincides with, the hydrolytic activity of other enzymes, especially β-1,3- and β-1,6 glucanases.

In the present study, the possibility that bacterial cell wall-degrading enzymes might be participated in inhibition and degradation of *Pythium* hyphae could be excluded for the following reasons: (a) 2,4-DAPG extraction procedure by organic solvents could eliminate any enzymatic activities that might be present in the bacterial culture, and (b) no β-glucanases activities could be detected in the supernatants of *P. fluorescens* Q2-87 even in the culture medium amended with laminarin and carboxymethylcellulose which is known to induce such enzymatic production for β-1,3 glucanase and cellulase, respectively (Winstead and McCombs 1961; Moore and Couch 1966; Janardhanan and Husain 1974). Thus, the source of these higher enzymatic activities, that caused growth inhibition and cell-wall degradation, was *Pythium* itself. However, extracellular hydrolytic enzymes by some *P.* spp., other than Q2-87 strain, are considered important determinants of the antagonistic ability of these bacteria against plant pathogens. The role of these enzymes in biocontrol is supposed to be connected to their direct action against plant pathogens (Fridlender et al. 1993). These authors showed that *Pseudomonas capricola* has the ability to inhibit several plant pathogens in the greenhouse including *Pythium ultimum, Fusarium*
oxysporum, Rhizoctonia solani, and Sclerotium rolfsii by such cell wall-degrading enzymes. Other studies also indicated that there is significant relationship between antagonistic activity of fluorescent Pseudomonas and the level of β-1,3 glucanase production which suggests that β-1,3 glucanase production may be responsible for the biological suppression of phytopathogens (Sneh et al. 1984; Jones et al. 1996; Velazhahan et al. 1999; Meena et al. 2001). The modes of action of P. fluorescens strains differ depending on the bacterial strain, as reported by Whipp et al.

The present results indicate that P. fluorescens Q2-87 was able to produce the phenolic antibiotic 2,4-DAGP under the experimental conditions used in this investigation. Growth inhibition and cell-wall degradation of Pythium might be attributed to 2,4-DAGP. Exogenous application of this antimicrobial compound to oomycete Pythium may influence the synthesis and/or activities of cell wall-polysaccharide-degrading enzymes, β-1,3 glucanase and cellulase activities that participated in cell-wall degradation. Further, β-glucanases that involved in degradation process does not originate from bacterial cells. Further work will be required to fully elucidate the modes of biocontrol agent P. fluorescens Q2-87 action of a major plant pathogen, Pythium. The interaction of other biological compounds with applied 2,4-DAGP may elucidate this mode of action.

REFERENCES


تأثير مضادات الحيوى النباتي من ب. فورمايونس فورميسن سلالة Q2-87
على إنزيمات البيتان 17 جلوتاتين والسيلابوتين في بيثيم allegedly
سحر على جمال الدين يوسف 1 - يامل أحمد حسين طرطوة 2 - مور الدبهان نصر
قزم فارجس
قسم النباتات الزراعية - جامعة قناة السويس بالأسماوية - مصر
قسم اليساكن - جامعة ميشيغان بأمريكا
قسم أمراض النبات - جامعة ميشيغان بأمريكا

في بحث سابق للمؤلفين تم استنتاج أن النتائج التأثري 2047 على أمبي مصبب فورميسن سلالة Q2-87 له قدرة على إحداث ارتفاع بيئي مسببة أمراض
للنباتات ودلالة هذه النتائج فسيولوجية تؤدي إلى تأثير نمو النباتات. وقد أجري
هذا البحث بهدف تأكيد قدرة تل. السلاسلة الببتيدية على إنتاج 2045 على أمبي مصبب فورميسن سلالة. وتظهر هذه النتائج لعمليات تكييفها وأيضا لتقييم بعض الأشباه الإزيمية، بيتا 17 جلوتاتين والسلاسلة، المسؤولة عن تحلل بيئيات المحمول خارجا تأثيرها في المحمول الحيوى المسئول من تل. السلاسلة.

أوضح النتائج المحتملة عليها باستخدام فورميسن سلالة الزكية ومواد تكييف
البيئية. في المراحل الأذآر عجر الجنس الحيوى Q2-84 على أمبي مصبب فورميسن سلالة ما في بيئات
بيئيات، كما أوضح النتائج أيضا أن هناك مستويات عالية لأشكال التآكل المائي البيئة
17 جلوتاتين والسمابوتين في بيئيات المعامل بمقاومة باليزول. وأن مصدر تل. الإزيمات هو
بيئيات من النتائج التي تم الحصول عليها تم اقتراح أن المحمول الحيوى للبيئيات هو المسؤولة
عن مقاومة بيئيات حيويا تتأثر بجدرها الخلايا السليمة وبيئات نمو الطبيبي.