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In vitro and *In vivo* Production of Pectolytic Enzymes by Some Phytopathogenic Fungi Isolated from Southwest Saudi Arabia

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Abstract. Five fungal plant pathogens isolated from crops grown in southwest Saudi Arabia were investigated for their *in vitro* and *in vivo* ability to produce pectolytic enzymes. Only *Alternaria macrospora* did not produce any appreciable amount of enzymes *in vivo* and *in vitro*. *Pythium debaryanum, Fusarium oxysporum, Rhizoctonia soluni, Fusarium equiseti* produced pectolytic enzymes *in vitro*. These same four fungi produced pectolytic enzymes *in vivo* which were detected after ten-fold concentration of blended infected host tissue. The pH values of peak enzyme activity of 4.0 and 5.0 indicate that the enzymes produced were polygalacturonases. The monogalacturonide end-product of pectin and cell wall degradation indicate that exopolygalacturonases were produced by the pathogens. High levels of *in vitro* endopolygalacturonaes, determined viscometrically, and the detection of low concentration of these enzymes in infected host plant tissues, and also the slow pectin-splitting action of the exopolygalacturonases produced, may account for the rot development and the wilt nature of the diseases these four fungi cause.

Introduction

Most phytopathogenic microorganisms produce enzymes capable of degrading the complex polysaccharides of the plant cell wall [1-3]. These enzymes are usually extracellular, stable and present in host plant tissues [4,5]. They are produced only by the soft rot-causing organisms [6]. Microorganisms causing dry, slowly-developing necrotic symptoms such as leaf spots, blotches, cankers, etc., do not produce significant amounts of cell wall degrading enzymes. With some bacterial plant pathogens causing necrotic symptoms, such as certain species of *Xanthomonas*, plant cell walls are degraded enzymatically, they are not degraded as rapidly as by the soft rot-causing organisms [6-9].

In this study we report on the production of pectolytic enzymes by

certain leaf spot and soft rot causing fungi in *vitro* and in *vivo* the type of enzyme produced and the end-product of the degradation of pectic substances by such organisms.

Materials and Methods

Source and growth condition of fungal isolates

The fungi used in this study were isolated from crops grown in Jizan region, southwest Saudi Arabia. The fungi were *Fusarium oxysporum* Sch. Ex. Fr., *Rhizoctonia solani* Kuhn, *Pythium debaryanum* Anct. non R. Hesse, *Fusarium equiseti* (Corda) Sacc. and *Alternaria macrospora* Zimm. Fungi were isolated from various plants, *F. oxysporum* from wilted leaves of watermelon (*Citrullus vulgaris* Schard ex Eckl and Zeth.), *R. solani* from roots of *carica papaya* Linn., *P. debaryanum* from infected tomato plants (*Lycopersicum esculentum* Mill.), *F. equiseti* from rotted fruits of banana (*Musa paradisiaca* Linn.), and *A macrospora* from dry necrotic leaves of okra (*Hibiscus esculentus* Linn.). The fungi were grown on potato dextrose agar (PDA) until a pure culture was established. The isolated fungi were identified according to Ellis [10], Plaats-Niternik [11], Saksena and Vaartija [12], and Nelson *et al.* [13].

Enzyme preparation and culture techniques

Pectic enzymes were obtained from the culture filtrate of the isolates, grown on a Dox medium containing 2 g of $NaNo_3$, 1 g of KH_2PO_4, 0.5 g of KCl, 0.5 g of $MgSo_4$. $7H_2O$ and 0.01 g of $FeSo_4$. $7H_2O$. Sucrose was omitted from the medium. Pectin N.F. (Sunkist Growers) at 1% concentration was used as the sole carbon source. The medium was buffered at desirable pH and was closely monitored (see below). Cultures were grown on a shaker at 25°C in the dark in 250 ml flasks containing 25 ml of medium, which had been autoclaved for 15 min at 120°C. fungal mycelia were removed at 2-day intervals, over 16 days, by centrifugation (10,000 xg for 20 min at 4°C), and the culture filtrate was dialyzed against several hundred volumes of distilled water for 18 h at 4 C. This filtrate was used immediately as a crude enzyme source or stored at -20°C until needed.

pH adjustments

The medium used for enzyme preparation was adjusted to the desired pH with 0.1M HCl for the low pH values and with 0.1 M NaOH for the high pH values. Thus, for media at pH 4.0 or 5.0, 100 mM citrate phosphate buffer was used while for pH 7.0 or 9.0, 50 mM Tris-HCl buffer was used, five replicates being prepared for each medium.

Enzyme assay

Endopolygalacturonase activity, based on change in the viscosity of the reaction mixture at 30°C, was determined using Ostwald-Fenske viscometer. Reaction mixture contained 20 ml of 1% pectin solution, buffered at pH 5.2 (0.1 M citrate), and 10 ml of enzyme solution. The substrate control contained distilled water in place of enzyme

solution while distilled water served as water control. Viscometric runs were made at 5 min. intervals for 20 min. Enzyme activity was expressed as the percent decrease in viscosity (D) and was calculated with the formula: $D = (100(T_s-T_t)/T_s-T_w)$ where T_s is the flow time of the substrate control. T_t is the flow time of the test and T_w is the flow time of water. Results are recorded as the % reduction in viscosity/10 min/mg protein.

Protein determination

The protein content of the enzyme preparations was estimated by the method of Lowry *et al.*, [14]. Crystalline bovine serum albumin was used as the standard.

Extraction of pectolytic enzymes

The *in vivo* assay to detect pectolytic enzymes produced in tissues infected by various fungi was carried out by cutting up the infected tissues and grounding 100 g of each sample in a mortar in liquid nitrogen. One gram of the tissue was taken up in 5 ml of cold 100 mM citrate phosphate (pH 4 and 5), or 50 mM Tris-HCl (pH 7 and 9) buffer. One milligram of polyvinylpyrrolidone (PVP) was added to 1 ml of buffer to adsorb phenols. The tissue was extracted in a Waring Blender for 2 min, strained through muslin and then centrifuged at 12,000 g for 20 min at 4°C. The supernatant was dialysed against distilled water for 12 h at 4°C, and used for the enzyme assays.

Detection of reaction products

The reaction mixture for the enzyme assay contained 5.0 ml of 0.5% pectin in 0.1 M citrate buffer at pH 5.2 and 5.0 ml of enzyme preparation. At each assay period a 50 ul aliquot of the reaction mixture was applied as a spot on a precooked silica gel TLC (thin layer chromatography) plate (0.25 mm). Ascending chromatography was used for the detection of reaction products in enzyme digests. The solvent used for separation contained 50 mg bromophenol blue and 60 mg sodium formate dissolved in a mixture of 85 ml of 77% (v/v) ethanol and 15 ml of 88% (v/v) formic acid. Following development for 4-6 h at 24°C, the plates were dried at 50°C for 24 h and visualized as yellow spots on a blue background.

Results and Discussion

A. macrospora did not produce any appreciable amount of pectolytic enzyme at the various pHs used (Fig. 1). The small amount detected had the highest activity at pH 4.0 and the lowest at pH 9.0. This fungus attacks okra plants and produces necrotic symptoms in comparison with the other fungi used in this study, which produce rots and wilts. It is surprising that *A. macrospora* did not produce polygalacturonase *in vitro* (Fig. 1) or *in vivo* (Fig. 6). It is possible that other enzymes such as pectin trans-eliminase, pectin methylesterase and cellulase not detected by the methods used are produced. It is also possible that cell wall degradation may not be the method by which host plant tissue is destroyed by *A. macrospora*. Phytotoxic, non-pectolytic substances are known to be

produced by pathogens which destroy plant tissues. An example of these substances is phytolysine produced by *dothedia ribesia* [15].

The time course of production of pectolytic enzymes by *Pythium debaryanum* is shown in Fig. 2. The enzyme activity at pH 4.0 was relatively low during the first 8 days of growth but then began to rise reaching a peak in 12-days and declined thereafter. At pH 5.0, the enzyme activity reached its peak in 4 days fell after 8 days and rose again on the 16th day of culture. The enzyme activity was low at pH 7.0 and reached its highest level on 16th day; at pH 9.0 the enzyme activity remained low for the 16 days of incubation. *P. debaryanum* causes damping off in tomatoes. The nature of the disease symptoms supports the involvement of pectolytic enzymes produced by this fungus. The data show that at pH 4.0, the 12-day old culture produces a high amount of pectolytic enzymes. Also, there is a peak of enzyme activity of pH 5.0, after 4 days of incubation. This early peak could have implications for pathogenecity and virulance.

Peak production of pectolytic enzymes by cultures of *F. oxysporum* at different pHs are shown in Fig. 3. At pH 4.0, the highest enzyme activity was found in four-day old culture, further culture caused a decline in the enzyme activity. At pH 5.0, the enzyme activity peaked in the 8-day old culture. At pHs 7.0 and 9.0 the enzyme activity remained low. *F. oxysporum* is known to cause wilt of water melon in southern Saudi Arabia [16] and has also been associated with wilt of tomato and okra [17,18]. The nature of the disease development again suggests that this fungus influences the host via production of pectolytic enzymes. It should be noted that at its peak, the enzyme production by *F. oxysporum* was greater than that by *P. debaryanum*.

The time course of pectolytic enzyme production by *R. solani* is shown in Fig. 4. Enzyme activity was detected in the crude preparations of two-day old cultures. The highest enzyme activity was detected in four-day old cultures at pH 4.0 and in 8 and 10-day old cultures at pH 5.0. The peak enzyme activity was lower and occurred at 10 days in cultures grown at pHs 7.0 and 9.0. *R. Solani* attacks *Carica papaya* and causes rot and wilt of roots [19]. It has also been associated with fruit rot of egg plant [20], wilt of sesame [21] and root rot of mango in Jizan [19]. The nature of these diseases suggests that *R. solani* produces pectolytic enzymes.

Fusarium equiseti produced pectolytic enzymes at pH 5.0 and two peaks of enzyme activity at 4 and 12 days (Fig. 5). At pH's of 4.0 and 7.0 the enzyme activity remained low for the first 8 days. At pH 4.0 the enzyme activity peaked at 12 days while at pH 7.0 the enzyme activity continued to rise until the 16th day. There was little enzyme activity when the fungus was grown at pH 9.0. *F. equiseti* causes fruit rot of banana in Jizan [19] and tuber rot of potato [22]. The fungus has also been associated with tomato [18] and fruit rot of grape [21]. This study shows that at pH 5.0, the 12-day old fungal culture produces a large amount of pectolytic enzymes. However the enzyme production was lower than that produced by *F. oxysporum, Pythium debaryanum* and *R. solani*. It is possible that several enzymes are produced and that cell wall degradation may not be the only method by which host plant tissus are destroyed by *F. equiseti*.

Species of *Fusarium* are known to produce the trichothecene group of toxins which are teragenic, carcinogenic and metagenic in animal tissues[23]. They may also affect plant tissues in the same or different way.

Pectolytic enzymes were detectable at various pHs in extracts from host tissues infected by all the fungi except *A. macrospora.* (Fig. 6). Tissues infected by *f. oxysporum* produced the highest enzyme activity followed by *R. solani, P. debaryanum* and *F. equiseti* in descending order. The highest enzyme activity of *F. oxysporum* among the fungi studied does not mean that *F. oxysporum* was the best producer of enzyme as compared to others because different fungi infected different types of tissues. The production of enzyme by fungi is dependent upon the type of tissues infected. The presence of pectolytic enzymes in infected host tissues suggest that they might be involved in the pathogenic process. The pectolytic enzyme activity was detected only after ten-fold concentration of blended host tissue indicating that the activity is very low and is not a cause of great damage. It is possible that, the pectolytic enzymes investigated are inhibited by compounds other than phenolics released during the extraction process as reported by Bergmann*et al.* [24].

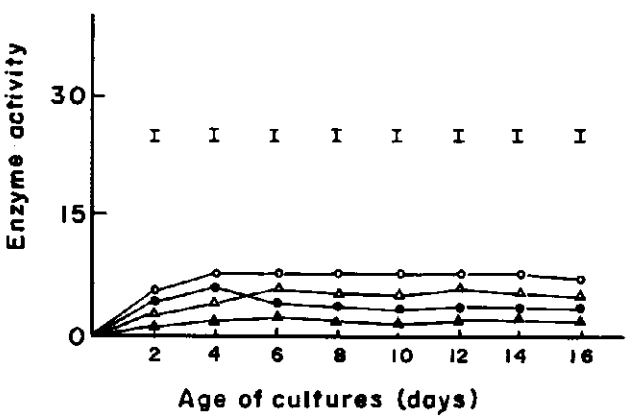

Fig. 1. Time course of production of pectolytic enzymes by *Alternaria macrospora.*
○ pH 4.0, ● pH 5.0, △ pH 7.0, ▲ pH 9.0
Bars represent LSD values (P< 0.05).

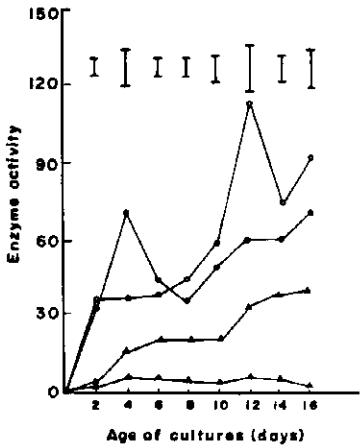

Fig. 2. Time course of production of pectolytic enzymes by *Pythium debaryanum*.
○ pH 4.0, ● pH 5.0, △ pH 7.0, ▲ pH 9.0
Bars represent LSD values (P< 0.05).

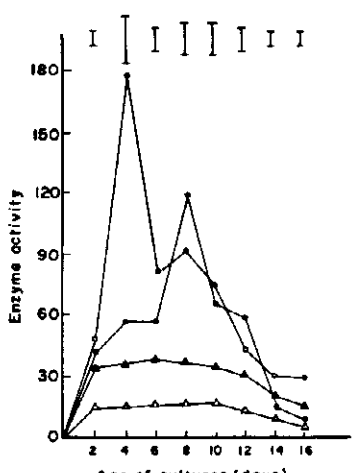

Fig. 3. Time course of production of pectolytic enzymes by *Fusarium oxysporum*..
○ pH 4.0, ● pH 5.0, △ pH 7.0, ▲ pH 9.0
Bars represent LSD values (P< 0.05).

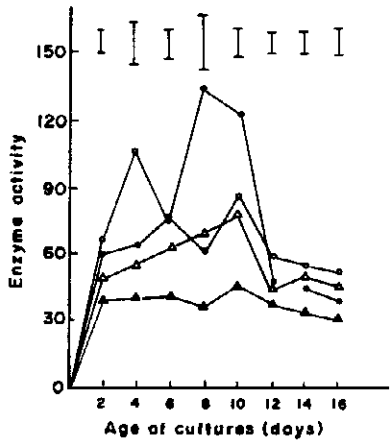

Fig. 4. Time course of production of pectolytic enzymes by *Rhizoctonia solani*.
○ pH 4.0, ● pH 5.0, △ pH 7.0, ▲ pH 9.0
Bars represent LSD values (P< 0.05).

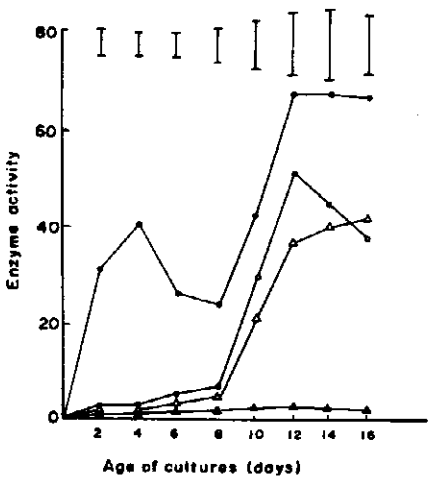

Fig. 5. Time course of production of pectolytic enzymes by *Fusarium equiseti*.
○ pH 4.0, ● pH 5.0, △ pH 7.0, ▲ pH 9.0
Bars represent LSD values (P< 0.05).

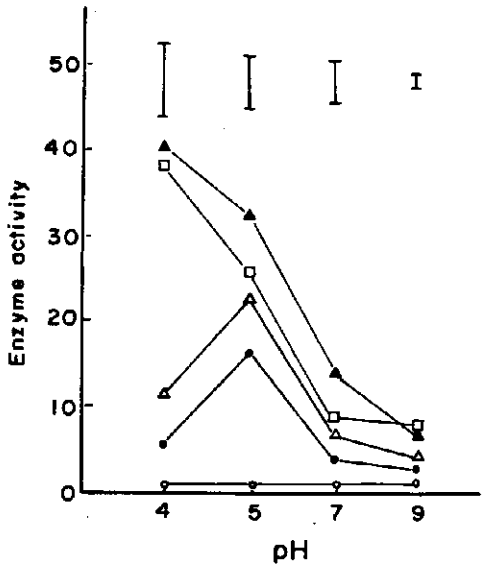

Fig. 6. Extraction of pectolytic enzymes from infected tissues at different pH values
▲ ***Fusarium oxysporum***, □ ***Rhizoctonia solani***, Δ ***Pythium debaryanum***, ● ***Fusarium equiseti***, ○ ***Alternaria macrospora***.
Bars denote LSD values (P< 0.05).

Very low pectolytic activity was detected in healthy plants not infected with the pathogens (Fig. 7). The highest activity was detected in tomato and the lowest in okra plants. Heale and Gupta [25] found that pectolytic enzyme levels were not as high in plant extracts as in pectin or polypectate grown fungal cultures, possibly because the tissues ground up during extraction included much uninfected and non-vascular tissue, plus the fact that there was almost certainly considerably less fungi present.

The low enzyme activity at pH 7.0 and 9.0 could mean that lyases are not involved in the pathogenetic process. Lyases are known to show their optimum activity at pH 8.0 and above [2,7,9,26,27]. The high enzyme activities at pH 4.0 and 5.0 suggest that the enzymes produced by the fungi both *in vitro* and *in vivo* are polygalacturonases (hydrolases). These enzymes are known to have their optimum activity at the low pH values [2,3,4,9,26]. The end product of degradation of pectic substrate *in vitro* (not shown) and *in vivo* detected by TLC was monogalacturonide (Rf. 0.52) (Fig.8). This indicates that exo-polygalacturonase enzymes are produced by the four fungi. Exo-enzymes split pectic chains at their ends, releasing monogalcturonide; hence their action is significant [2, 8, 28]. This coupled with the high concentration of endopoly-

galacturonases, particularly *in vitro*, could be responsible for the rot and wilt caused by these pathogens with the exception of *A. macrospora* which produced no polygalacturonase.

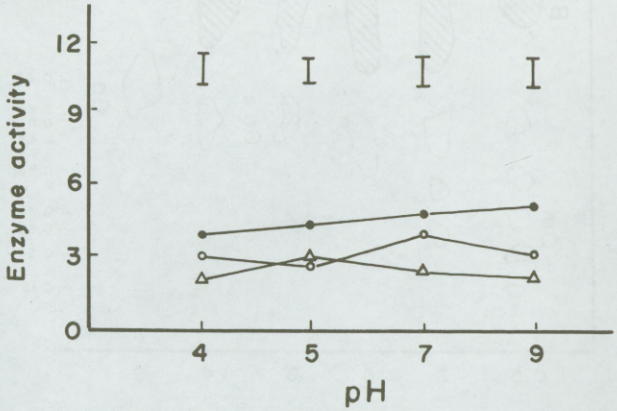

Fig. 7. Activity of the enzyme extracted from healthy tissues.
● Tomato, ○ Papaya, △ Okra
Bars denote LSD values (P< 0.05).

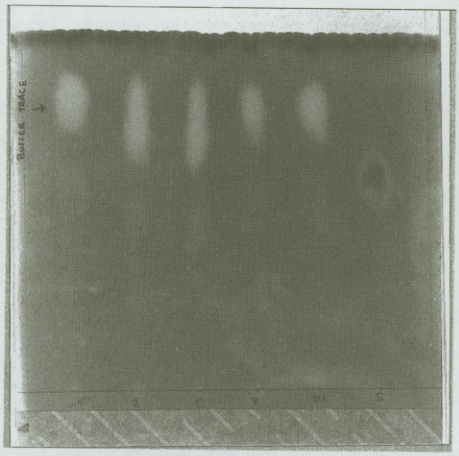

Fig. 8a. Thin layer chromatogram showing the reaction products from pectin released by the action of pectic enzymes prepared from *P. debaryanum* infected tomato tissues. Ten-fold concentration of blended infected tomato tissue were used.

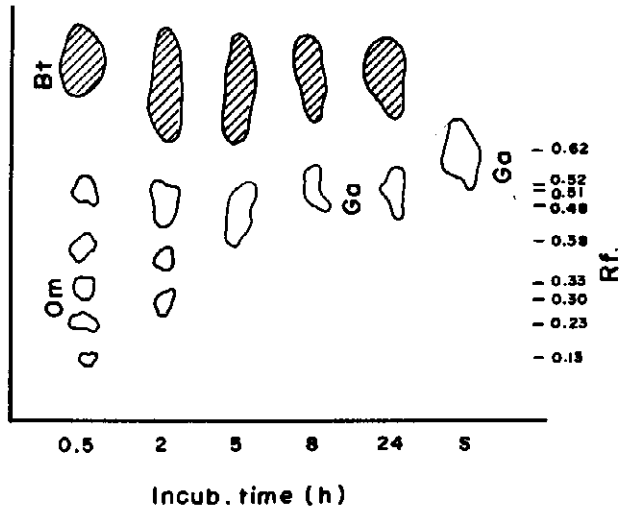

Fig. 8b. Diagrammatic representation of *in vivo* action of pectic enzymes prepared from *P. debaryanum* infected tissues.
S: standard galacturonic acid (100 µg/ml); Om: oligomers; Ga: galacturonic acid; Bt: represents trace buffer.

References

[1] Wood, R.K.S. "Specificity in Plant Diseases." In: *Fungal Pathogenicity and the Plants Response.* Byrde. R.J.W. and Cuttings, C.V. (Eds.), London: Academic Press, 1973, pp. 1-16.

[2] Bahkali, A.H. "Specificities of Pectolytic Enzymes Produced by *Verticillium tricorpus.*" *Tran. Mycol. Soc. Japan.*, 30 (1989), 161-167.

[3] Bahkali, A.H. "*In vitro* Production of Pectolytic and Cellulolytic Enzymes by *Colletotrichum lindemuthianum* Isolated from Soybean Grown in Saudi Arabia." *World J. Microbiol. Biotech.* 8 (1992), 55-59.

[4] Bateman, D.F. and basham, H.G. "Degradation of Plant Cell Walls and Membranes by Microbial Enzymes. In Physiological Plant Pathology:. Heitefuss, R. and Williams, P.H. (Eds.). *Encyclopaedia of Plant Physiology*, vol. 4, Berlin: Springer-Verlag, 1976, pp. 316-355.

[5] Bahkali, A.H. "Degradation of Date Palm (*Phoenix dactylifera* L.) Cell Walls by Extracellular Pectolytic Enzymes Produced by *Verticillium alboatrum* and *Verticillium dahliae.*" *Int. J. Tropical plant diseases.* 5 (1987), 165-172.

[6] Christinc, R., Freyssinet, G. and Fevre, M. "Production of Cell Wall-degrading Enzymes by the Phytopathogenic Fungus *Scierotinia sclerotiorum.*" *App. Environ. Microbiol.*, 57 (1991), 1478-1484.

[7] Dusterhoft, E.M., Bonte, A.W., Venekamp, J.C. and Voragen, A.G.J. "The Role of fungal Polysaccharidases in the Hydrolysis of Cell Wall materials from Sunflower and Palm-kernel meals."

World J. Microbiol. Biotechnol., 9 (1993), 544-554.

[8] Gainvors, A., Frezier, V., Lemaresquier,H., Lequart, C., Algle, M. and Belarbi, A."Detection of Polygalacturonase, Pectin-lyase and Pectinesterase Activities in a *Saccharomyces cerevisiae* Strain." *Yeast*, 10 (1994), 1311-1319.

[9] Aljohimi, I.M. "Properties and Characterization of Polysaccharidases Complex of Fungi Associated with Soft-rot Fruits and Vegetables in Riyadh." *M.Sc. Thesis, King Saud University* 1995.

[10] Ellis, M.B. "Dematiaceous hyphomycetes." Surrey, England: C.M.I. Kew, 1971.

[11] Plaats-Niterink, A.J. "Monograph of the Genus *Pythium*..." C.B.S. Holland 1981.

[12] Saksena, H.K. and Vaartija, O. "Description of New Species of *rhizoctonia*." *Can. J. Bot.*, 38 (1960), 931-943.

[13] Nelson, P.E., Toussoum, T.A. and Marassas, W.O. "An Illustrated manual for Identification.*Fusarium species.*" The Pennsylvania State University Press, 1983.

[14] Lowry, C.H., Rosebrough, N.J. Farr, A.L. and Randall, R.J. "Protein Measurement with the Folin Phenol Reagent." *J. Biol. Chem.*, 193 (1951), 265-275.

[15] Naef-Roth, S., Gauman, E. and Albersheim, P. "Phytolysine, a Non-enzyme Tissue Degradant Produced by *Dothedia ribesa*." *Phytopathol. Z.*, 40 (1961), 283-302.

[16] Kassim, M.Y.; Bokhary, H.A. and Abou-Heilah, A.N. "General Survey of Plant Diseases and Pathogenic Organism." *Saudi Biol. Soc.*, 1989.

[17] Abu-Yaman, I.K. and Abu-Blan, H.A. "Major Diseases of Cultivated Crops in the Central Province of Saudi Arabia." *I. Diseases of Fruit Trees. Z. Pflan. Z. Kh. Pflan. Z. Sch.*, 78 (1971), 607-611.

[18] Abou-Hellah, A.N., Kassim, M.Y., Sheir, H.M. and Shamsher, K. "Survey of Fungal Diseases in Saudi Arabia. 1. Diseases of Vegetables." *Comm Agric. Sci. Dev. Res. Coll. Agri. Univ*, Alexandria, Egypt. 4 (1983), 16-28.

[19] Abu-Saleh, H.S., and Munawar, M.N. "Prevalent Agricultural Pests Distributed on Vegetable, Crops and Fruit Trees in Jizan Area." *Min. Agric. And Water, Reg. Agric. Water Res.* Center, Riyadh, Saudi Arabia (sponsored by F.A.O.) (1987), pp. 15-30 (in Arabic).

[20] Basalah, M.O., Bahkali, A.H. and Sher, M. "Peroxidase Activity in Extracts of Egg-plant (*Solanum melongena* L.) Fruits Inoculated with Isolates of Some Fungi." *Bot. Bull. Academia Sinica.*, 29 (1988), 275-279.

[21] Kassim, M.Y., Abou-Heilab, A.N., Sheir, H.M. and Shamsher, K. "Survey of Fungal Plant Diseases in Saudi Arabia." 2. "Diseases of Fruit Trees and Field Crops." *Comm. Agric. Sci. Dev. Res.*, 4 (1983), 29-43.

[22] Abu-Thuraya, N.H. "General Survey of Agricultural Pests in Saudi Arabia." *Agric. Res. Dept., Pl. Prot. Res. Div., Min. Agric and Water*, Riyadh (1982), 240 pp (in Arabia).

[23] Sorenson, W.G., Simpson, J. and Castranova, V. "Toxicity of the Mycotoxin Patulin for Rat Alveolar Macrophages." *Environ. Res.*, 38 (1985), 407-416.

[24] Bergmann, C.W., Ito, Y., Singer, D., Albersheim, P., Darvill, A.G., Benhamou, N., Nuss, L., Salvi, G. Cervone, F. and Delorenzo, G. "Polygalacturonase-inhibiting Protein Accumulates in *Phascolus vulgaris* L. in Response to Wounding, Elicitors and Fungal Infection." *Plant Journal*, 5 (1994), 625-634.

[25] Heale, J.B. and Gupta, D.P. "Mechanism of Vascular Wilting Induced by *Verticillium alboatrum.*" *Trans. Br. My. Soc.*, 58 (1972), 19-28.

[26] Cooper, R.M. and Wood, R.K.S. "Cell Wall-degrading Enzymes of Vascular Wilt fungi." III. Possible Involvement of Endo Pectin Lyase in *Verticillium* Wilt of Tomato. *Physiol. Pl. Pathol.*, 16 (1980), 285-300.

[27] Bahkali, A.H. "Studies of the Pectic Enzyme complex Produced by *Verticillium* Species." *Ph.D. Thesis*, University of Wales, U.K. 1983.

[28] Cooper, R.M. "Regulation of Synthesis of Cell Wall Degrading Enzymes of *Plant Pathogens*." In: *Cell Wall Biochemistry Related to Specificity in Host-Plant Pathogen Interactions*. Solheim, B. and Raa. J. (Eds.). Oslo: Universitetsforlaget, 1976, pp. 163-211

إنتاج الأنزيمات البكتوليتية في المعمل وفي أنسجة النبات المصابة بوساطة بعض الفطريات الممرضة المعزولة من جنوب غربي المملكة العربية السعودية

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(استلم في ٦/ ٧/ ١٤١٦هـ، وقبل للنشر في ٢١/ ٧/ ١٤١٧هـ)

ملخص البحث. تفرز معظم الكائنات الحيّة الممرضة للنبات أنزيمات لها القدرة على تحليل المركّبات عديدة التسكر المكوّنة للجدار الخلوي في النبات. عادةً ما تكون هذه الأنزيمات خارج خلوية وثابتة عند استخلاصها من أنسجة النباتات المصابة وكثيرًا ما يعتقد أن الكائنات المسببة للعفن الرخو أكثر الكائنات إفرازًا لمثل هذه الأنزيمات نتيجة للطبيعة السريعة في تحلل الأنسجة الخلوية، أما الكائنات المسببة لأعراض التحلل الموضعي وأعراض العفن الجاف والبطيئة في تحللها للأنسجة فإنها لا تفرز تلك الأنزيمات المحلّلة للجدر الخلوية. على هذا الأساس أجريت هذه الدراسة على خمسة فطريات ممرضة لنباتات المحاصيل في المنطقة الجنوبية الغربية بالمملكة العربية السعودية للتأكد من قدرتها على إفراز الإنزيمات البكتينية المحللة للجدر الخلوية.

أثبتت نتائج الدراسة أن فطرة *Alternaria macrospora* والمسببة للتحلل الموضعي الجاف في أوراق الباميا لا تنتج الأنزيمات البكتينية بكميات كبيرة، سواءً معمليا أو في مستخلصات النبات المصابة، وفي المقابل نجد أن فطريات *Fusarium oxysporum, Pythium debaryanum, Fusarium equiseti, Rhizoctonia solani* تنتج الأنزيمات البكتينية معمليا، كما تنتجها أيضًا في مستخلصات الأنسجة المصابة، في حين أن هذه الأنزيمات لا يمكن الكشف عنها إلا بعد تركيزها عشر مرات، كما أن الأرقام الهيدروجينية PH للنشاط الأنزيمي الأمثل كانت عند ٤, ٥، وهذا دليل واضح على أن الأنزيمات المفرزة أنزيمات polygalacturonases في طبيعتها. كما أجريت الدراسة على الجلاكتورونات الأحادية monogalacturonides للنواتج النهائية لتحلل البكتين والجدر الخلوية للأنسجة النباتية والتي تشير إلى إفراز أنزيم exopolygalacturonase بوساطة الفطريات المستخدمة.

وحيث إنه أمكن استخلاص الأنزيمات البكتينية من أنسجة النبات العائل المصاب، وكذلك عمل أنزيم exopolygalacturonase على تكسير البكتين لدليل على الإصابة بالذبول التي تحدثها الفطريات الأربعة قيد الدراسة والمنتجة للأنزيمات البكتينية.