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Inducing Resistance in Potato Plants against Late Blight Disease in Relation to Elicitation of Phytoalexins

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Late blight disease of potato caused by *Phytophthora infestans* (Mont.) de Bary is one of the most devastating diseases of potatoes all over the world and in some regions in Egypt.

Coconut milk solution (2%), mango malformed inflorescence extract (7.5%), ethyl salicylic acid (0.125 ml/liter), Sincocin and Agrispon (0.1%) led to a great reduction in number of blight spots/ plant in comparison to non treated plants.

All tested agents reduced spot size especially ethyl salicylic acid as it reduced spot size from 11.2±1.05 mm in non-treated plants to 2.7±2.12 mm in the treated ones.

Disease severity was reduced in treated plants where the best solutions were jasmonic acid and mango malformed inflorescence extract, which reduced disease severity from 50% on control plants to 1% in the treated ones.

All agents elicited sesquiterpenoid phytoalexins (SSP) in potato tubers. The number of SSP induced varied between tested agents, whereas, jasmonic acid elicited 5 compounds, mango malformed inflorescence elicited 2 compounds. All tested agents elicited lubimin and phytuberin.

Correlation coefficient between number of elicited SSP and disease severity reached-0.64 indicating that disease severity decreased by increasing number of elicited SSP.

Keywords: Agrispon, coconut milk, ethyl salicylic acid, jasmonic acid, mango malformed inflorescence, *Phytophthora infestans*, potato late blight and Sincocin.

Phytophthora infestans (Mont.) de Bary causes late blight disease of potato and tomato throughout the world. This devastating disease causes severe losses in potato fields every year if the disease was left without control (James, 1974). For a long time, the control of such serious disease was going in two directions. The first one was through using suitable fungicides, *i.e.* Mancozeb, Metiram, Benzamide, Cymoxyanils, Dimethomorphs, Azoxystrobin, Chlorothalonil, Coppers, Mefanoxams, Ridomil (metalaxyl) and other chemicals (Stevenson, 1993). The other was breeding for resistant cultivars against the most dominant races of the fungus (Fry, 1978; Wastie *et al.*, 1993 and Bradshaw *et al.*, 1995).

It is well established that usage of fungicides for a long time causes disturbance in ecosystem and might led to the appearance of fungicide-resistant isolates (Goodwin and McGrath, 1995 and Suijkowski *et al.*, 1995) in addition to their hazardous effects on humans and animals (Paris-Palacios *et al.*, 1998). However, dynamic population of *Phytophthora infestans* led to the appearance of new races which could infect the resistant cultivars (Fry and Goodwin, 1995; Suijkowski *et al.*, 1996 and Fry and Goodwin, 1997).

For all of these reasons, the concept of inducing resistance in plants against fungal, bacterial, viral and nematodal diseases became widespread in agriculture (Naylor *et al.*, 1998; Oka *et al.*, 1999; Buonaurio *et al.*, 2002; Mohan Babu *et al.*, 2002 and Bach *et al.*, 2003).

The present investigation aimed to induce resistance in potato plants against late blight disease under field conditions by some plant extracts, *i.e.* Coconut milk solution, mango malformed inflorescence extract, Sincocin and Agrispon and chemical compounds, *i.e.* ethyl salicylic acid and jasmonic acid which were chosen according to their ability to enhance soluble peroxidase activity in potato tubers. Studying the ability of the tested agents to elicit phytoalexins in potato tubers was also undertaken.

Materials and Methods

Field location:

A field (1/2 feddan) located in El-Sabeel village, Shebeen El-Kanater, Qualubiya Governorate was chosen to carry out this experiment because it has a long history in heavy epidemic of late-blight of potato (Mohamed, 2002). Field was divided into plots (7 x 6 m), every plot contained 7 lines. Every plot was specified for one tested agent and three plots were left as check control. Large area around the plots was left without treatment to avoid any contamination by any treated chemicals from nearly fields. Lines were sown by tuber pieces (cv. Dimond) in 5th of November, 2005. Experimental field was fertilized and irrigated as usual.

Tested agents:

The following plant water extracts and solutions were sprayed on plants:

1- Hot-water extract of mango malformed inflorescence (75 g/l.)
2- Water solution of coconut milk (2%).
3- Water solution of ethyl salicylic acid (0.125 ml/l).
4- Water solution of jasmonic acid (0.125 ml/l).
5- Water solution of Agrispon® (Plant and mineral extracts, commercial product obtained from Agric. Sci., Dallas Company (1 ml/ l).
6- Water solution of Sincocin® (Plant extracts, commercial product obtained from Agric. Sci., Dallas Company (1 ml/ l).

Plants were sprayed 4 times starting when they reached 60 days from planting (5th January 2006) and every 10 days.

Disease assessment:

The number of spots was counted on 20 plants distributed in 5 rows at the first appearance of late blight symptoms (28th January, 2006). The diameter (mm) of spots was measured. Three weeks later, when the lesions had coalesced, proportion of blighted area / leaf was visually estimated using a 0-4 scale (0= no symptoms; 1,2,3, and 4 up to 1%, 10%, 25% and 50% of the leaf area blighted, respectively (James, 1971).

Elicitation and chromatographic separation of sesquiterpenoid phytoalexins (SSP):

Elicitation of sesquiterpenoid phytoalexins (SSP) in potato tubers was carried out according to the method adopted by Metlitski *et al.* (1976). Newly harvested potato tubers were collected from treated and non-treated plots, washed under tap water to remove the adhesive soil particles then air-dried. Tubers were stored for 3 weeks under cooling (6-8°C), then transferred and kept at room temperature (25±2°C for 48h. Tubers were cut transversely into two equal portions and by using teaspoon, a hole (2cm in diameter, 1cm in depth) was digged in the centre of paranchymatous tissue, then washed with sterilized distilled water and left to dry on filter papers. Two hours later, holes were filled with water solutions of the tested agents or distilled water as a check. Ten halls were used for every particular solution. In order to avoid any fungal or bacterial contaminations, water solutions were amended with 500 ppm chloramphenicol+10 ppm benomyl. Halves of tubers were put in stainless steel plates covered with glass plate and incubated at 22±°C in the dark. Halls were refilled with sterilized distilled water when needed. Forty eight hours later, suspension of each agent was harvested, collected together then mixed with chloroform (1: 5v/v). Mixture was shaked well in a separating funnel and chloroform phase was separated, evaporated till dryness in a beaker under vacuum. The residue was re-dissolved in 0.5 ml chloroform.

Twenty microliters of chloroform containing residues were spotted on Silica-gel-G plates, 0.2 mm (Polygram®) for thin layer chromatographic separation. Separation was carried out using system consisted of chloroform: acetone (7:3 v/v). After running had been completed, the plate was heat dried then sprayed with vanilin-sulfuric acid reagent, and heated at 100°C for 5 minutes. Rf values of separated spots were calculated and compared with Rf(s) values of potato phytoalexins adopted by Stoessl (1982).

Statistical analysis:

Standard deviation for all treatments was calculated according to Michael (1984); Pierson correlation coefficient was calculated between all figures of disease incidence and number of sesquiterpenoid phytoalexins, elicited by all tested agents.

Results

Effect of chemical agents and natural extracts on late blight incidence:

Twenty plants distributed in 5 rows were chosen at the first appearance of late blight symptoms on potato plants (28/1/2006) to count number of spots/ plant for every particular treatment. The average numbers of late blight spot / plant on non-treated plants reached 8.12±4.16 spots/ plant. The average spot number was 1.25±1.45 on plants treated by coconut milk solution while it was 1.25±1.78 on plants treated by mango inflorescence extract. Significant differences in late blight spots/plant were found between plants treated by the previously mentioned agents and the control. On the other hand, no significant differences were recorded in number of spots/plant on plants treated with Agrispon, Sincocin and ethyl salicylic (Fig. 1). It is also clear from Fig (1) that jasmonic acid was the least effective agent in reducing spot numbers/ plant (5.87±4.32). Average spot diameter was reduced due to the treatment in comparison to the control. Jasmonic acid and ethyl salicylic acid solutions were highly effective in this respect comparing with other treatments (Fig. 2). In all cases, average spot sizes were less than that on control plants.

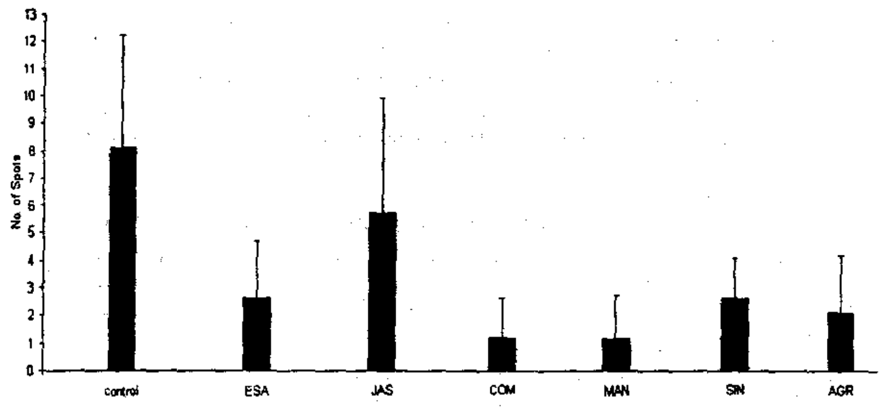

Fig. 1. Effect of chemical agents and natural extracts on number of late blight spots / plant.

Whereas: ESA: Water solution of ethyl salicylic acid (0.125 ml/liter).JAS: Water solution of Jasmonic acid (0.125 ml/l), COM: Water solution of coconut milk (2%). MAN: Water extract of mango malformed inflorescence (75 g/l). SIN: Water solution of Sincocin®(1 ml/l). AGR: Water solution of Agrispon(1ml/l).

Three weeks later, severity of late blight was determined on the chosen plants. Data in Fig. (3) indicate that non treated plants recorded maximum readings of disease severity (50%). On the contrary, coconut milk solution and mango malformed inflorescence extract gave the minimum score of disease severity (1%). Plants treated by Agrispon and Sincocin solutions recorded 5% disease severity, ethyl salicylic acid and jasmonic acid recorded 10% disease severity.

Phytoalexin induction in potato tuber tissues by the tested agents:

The ability of the tested agents to elicit phytoalexins in potato tuber tissues was tested using diffusion technique. Results of this study are presented in Table (1) and Fig. (4). It is clearly shown in Fig. (4) that all the tested agents elicited phytoalexins in potato tubers. It was also observed that, the ability of the tested agents as elicitors of phytoalexins was variable.

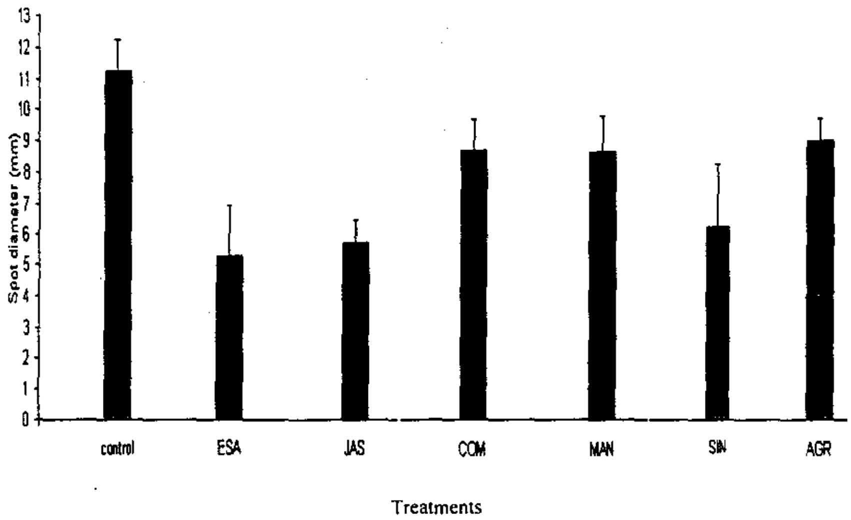

Fig. 2. Effect of chemical agents and natural extracts on size of late blight spots.
For treatments, refer to footnote of Fig. 1.

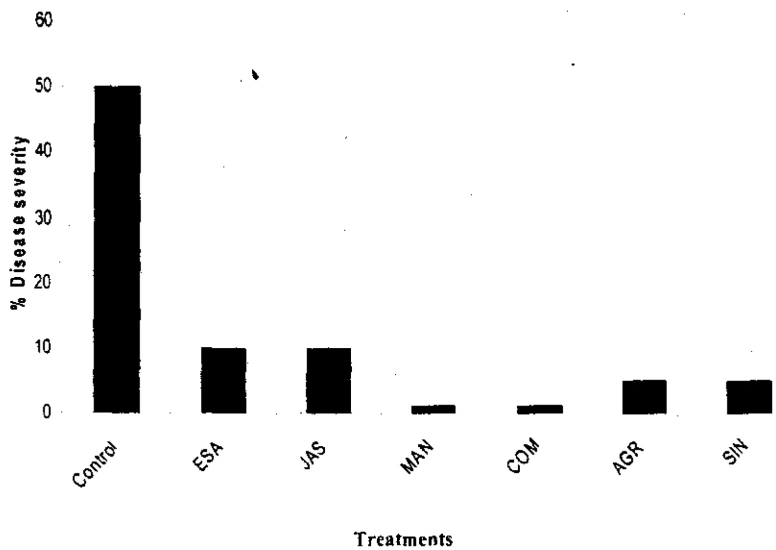

Fig. 3. Final late bight disease severity on potato plants.
For treatments, refer to footnote of Fig. 1.

Table 1. Phytoalexins elicited by tested agents, their Rf values and their name

Rf	Name of phytoalexin	Elicited by
0.046	Unknown	Jasmonic acid, coconut milk
0.093	Unknown	Jasmonic acid
0.157	Unknown	Ethyl salicylic acid, Jasmonic, Sincocin, Agrispon
0.203	Rishitin	Sincocin
0.509	Lubimin	All tested agents
0.824	Phytuberin	All tested agents

Table (1) also clearly show that all agents elicited lubimin and phytuberin. Moreover, unknown sesquiterpenoid phytoalexin compounds were elicited by jasmonic acid, coconut milk, ethyl salicylic acid, Sincocin® and Agrispon. The phytoalexin rishtin was only elicited by Sincocin.

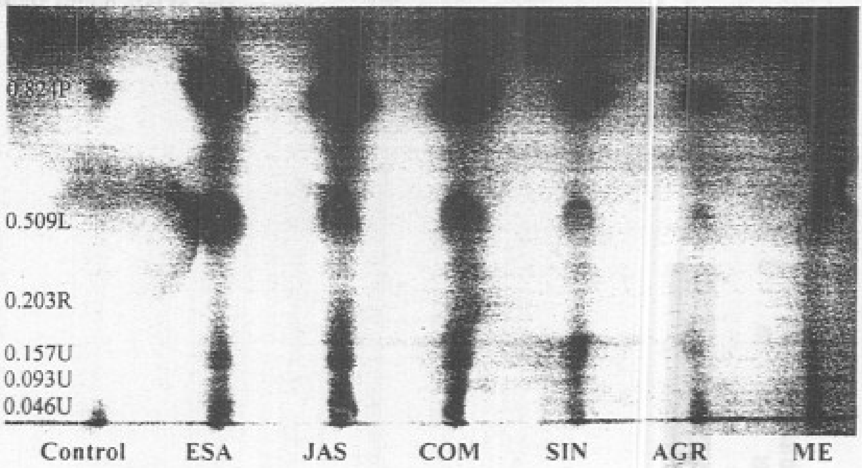

Fig. 4. Phytoalexins elicited by tested agents.

Whereas: P: Phytuberin; L: Lubimin; R: Rishitin; U: Unknown; ESA: Water solution of ethyl salicylic acid (0.125 ml/l); JAS: Water solution of Jasmonic acid (0.125 ml/l); COM: Water solution of coconut milk (2%); MAN: Water extract of mango malformed inflorescence (75 g/l.); SIN: Water solution of Sincocin® (1ml/l) and AGR: Water solution of Agrispon® (1 ml/ l).

Pierson correlation coefficient between numbers of sesquiterpenoid phytoalexins elicited by different agents and all of disease incidence figures was calculated (Table 2).

Table 2. Pierson correlation coefficient between number of sesquiterpenoid phytoalexins (SSP) elicited by different agent and disease incidence figures

State	Correlation r coefficient
Average number of spots/ plant x No. of SSP	-0.14
Average spot size/ plant x No. of SSP	-0.21
Average disease severity of No. of SSP	-0.68

It is also clearly shown in Table (2) that, high negative coefficient was found only between average disease severity and No. of SSP elicited by different agents, whereas it reached -0.68.

Discussion

The concept of inducing resistance of plants against disease was introduced vigorously in plant disease management. There are many successful trials carried out under laboratory and greenhouse conditions to enhance plant disease resistance against fungal diseases (Cohen *et al.*, 1991; Cohen, 1994 and Agostini *et al.*, 2003) bacterial diseases (Buonaurio *et al.*, 2002), viral diseases (Anfoka, 2000) and even nematodal diseases (Oka *et al.*, 1999), using different chemical agents.

Recently, different studies were carried out to enhance plant resistance against diseases by plant extracts (Daayf *et al.*, 2000 and Baysol *et al.*, 2002). In the present investigation, plant water extract of malformed mango inflorescence, water solution of coconut milk, Agrispon® and Sincocin® and chemical compounds, *i.e.* ethyl salicylic acid and jasmonic acid were tested as inducers of resistance against late blight disease, one of the most destructive diseases of potato under field conditions

Data obtained indicated that, all the tested compounds have reduced disease incidence. The best agents reducing number of spots/ plant were coconut milk solution and mango malformed inflorescence extract, meanwhile, the best agents reducing spot diameter were jasmonic acid and ethyl salicylic acid solutions. Disease severity was also reduced due to the treatment and the best agents were coconut milk solution and mango malformed inflorescence extract followed by Sincocin® and Agrispon® solutions.

Jasmonic acid and some derivatives of salicylic acid were found to induce resistance in plants against diseases via elicitation of pathogenesis related proteins (PR) (Cohen *et al.*, 1993; Vernooij *et al.*, 1995 and Van Wees *et al.*, 2000).

Potato tubers had proved their efficiency as a test organ for elicitation of phytoalexins. It was proved that any agent that elicit phytoalexins in potato tubers effectively elicit such phytoalexins in plant shoots (Mostafa and Djakov, 1977; Bostock *et al.*, 1982 and Szafrauek *et al.*, 2005). In this study the ability of the previously mentioned agents to elicit phytoalexins in potato tubers (cv. Diamond) was tested.

Data obtained also indicated that all tested agents elicited sesquiterpenoid phytoalexins (SSP) in potato tubers. Surprisingly, agents varied in their ability to elicit SSP. Some agents elicited five SSP compounds, (*i.e.* jasmonic acid) others, elicited two SSP compounds, (*i.e.* mango malformed inflorescence extract), but all agents elicited at least lubimin and phytuberin.

The role of phytoalexins in plant disease resistance was studied in many laboratories (Paxton, 1981; Bostock *et al.*, 1982Cohen *et al.*, 1991 and Ning *et al.*, 2003) and it was proved that there is a high correlation between elicitation of phytoalexins in inoculated plants and their resistance to the pathogens. In this respect, some studies were carried out to prepare phytoalexins elicitor and used it as a green pesticide (Ning *et al.*, 2003).

As a conclusion, jasmonic acid, ethyl salicylic acid solution, coconut milk, Agrispon®, Sincocin® solutions and mango malformed inflorescence extract had proved their efficacy as inducers of resistance agents against late blight disease of potato under field conditions by elicitation of phytoalexins in treated plants.

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Reference

Agostini, J.P.; Buchong, P.M. and Timmor, L.W. 2003. Greenhouse evaluation of products that induce host resistance for control of scab, melanose and Alternaria brown spot of citrus. *Plant Dis.*, **87**: 69-74.

Anfoka, G.H. 2000. Benzo-(1,2,2)-thiadiazole-7- carbothioic acid S-methyl ester induces systemic resistance in tomato (*Lyopersicon esculentum* Mill cv. Vollendung) to cucumber mosaic virus. *Crop Protection*, **19**: 401-405.

Bach, E.E.; Barros, B.C. and Kimati, H. 2003. Induced resistance against *Bipolaris bicolour, Bipolaris sorokiniana* and *Drechslera tritici repentis* in wheat leaves by xantham gum and heat inactivated conidial suspension. *J. Phytopathol.*, **151**: 411-418.

Baysol, O.; Laux, P. and Zeller, W. 2002. Further studies on the induced resistance (IR) effect of plant extract from *Hedera helix* against fire blight (*Erwinia amylovora*). *Acta Horticulturae*, **590**: 272-277.

Bostock, R.M.; Lane, R.A. and Kuc, J.A. 1982. Factors affecting the elicitation of sesquiterpenoids phytoalexins accumulation by eicosapentaenoic and arachidonic acid in potato. *Plant Physiol.*, **70**: 1417-1424.

Bradshaw, J.E.; Wastie, R.L.; Stewart, H.E. and Mackay, G.R. 1995. Breeding for resistance tc late blight in Scotland. In: *Phytophthora infestans*. L.J. Dowley, E. Bannon, L.R. Cooke, T. Keanen and E. O'Sullivan (eds.). Bude Press Ltd., Dublin, Ireland. 150pp.

Buonaurio, R. Scarponis, L.; Ferrara, M.; Sidott, P. and Bertona, A. 2002. Induction of systemic acquired resistance in pepper plants by acibenzolar-S. methyl against bacterial spot disease. *Europ. J. Plant Pathol.*, **108:** 41-49.

Cohen, Y. 1994. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by DL-3-amino-n-butyric acids. *Phytopathology*, **84:** 55-59.

Cohen, Y.; Gisi, U. and Mosinger, E. 1991. Systemic resistance of potato against *Phytophthora infestans* by unsaturated fatty acids. *Physiol. and Mol. Plant Pathol.*, **83:** 255-263.

Cohen, Y.; Gisi, U. and Nidrman, T. 1993. Local and systemic protection against *Phytophthora infestans* in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology*, **83:** 1054-1062.

Daayf, F.; Ongena, M.; Boiulanger, R.; El-Hadrami, I. and Belanger, R.R. 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew infected plants with extracts of *Reynoutria sacchalinensis*. *J. Chemical Ecol.*, **26**: 1579-1593.

Fry, W.E. 1978. Quantification of genera; resistance of potato cultivars and fungicide effects for integrated control of potato late blight. *Phytopathology*. **68:** 1650-1655.

Fry, W.E. and Goodwin, S.B. 1995. Recent migration of *Phytophthora infestans*. Pages: 89-95. In: *Phytophthora infestans*. L.J. Dowley, E. Bannon, L.R. Cooke, T. Keane. and O'sullivan (eds.). Bude Press Ltd., Dublin, Ireland. 150pp.

Fry, W.E. and Goodwin, S.B. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Dis.*, **81**: 1349-1357.

Goodwin, S.B. and McGrath, M.T. 1995. Insensitivity to metalaxyl among isolates of *Phytophthora erythroseptica* causing pink rot of potato in New York. *Plant Dis.*, **79:** 967.

James, W.C. 1971. *A Manual of Assessment Keys for Plant Diseases*. St Paul, MN, USA.

Metlitski, L.V.; Ozeretskovskaia O.L.; Yorganova, L.N.; Savelova, I.N. and Diakov, U.T. 1976. Inducing phytoalexins in potato tubers by metabolites of *Phytophthora infestans* (Mont.) de Bary. *Reports of Academy of Science, USSR*, **226:** 1217-1220.

Michael, P. 1984. *Ecological Methods for Field and Laboratory Investigations*. McGraw-Hill Publishing Company Limited, New York.

Mohamed, M.A.F. 2002. Forecasting of potato late blight under the Egyptian environmental conditions. M.Sc. Thesis, Plant Pathol. Dept., Fac. Agric., Ain Shams Univ., 111 pp.

Mohan Babu, R.; Sajeena, A.; Vijaya Samyundeeswari, A.; Spreedhar, A.; Vidyasekaran, P. Seetharman, K. and Reddy, M.S. 2002. Induction of systemic resistance to *Xanthomonus oryzae* pv. oryzae by salicylic acid in *Oryza sativa* (L.). *J. Plant Dis. and Protection,* **110:** 419-431.

Mostafa, M.H. and Djakov, U.T. 1977. Effect of systemic pesticides on interrelationship of *Phytophthora infestans* (Mont.) de Bary and hosts. Proc. 5th Inter. Symp. of systemic fungicides (Berlin), pp. 31-38.

Naylor, M.; Murphy, A.M.; Berry, J.O. and Carr, J.P. 1998. Salicylic acid can induce resistance to plant virus movement. *Molecular Plant-Microbe Infestations.* **11:** 880-868.

Ning, J.; Kong, F.; Lin, B. and Lin, H. 2003. Large scale preparation of the phytoalexin elicitor glucohexatose and its application as a green pesticide. *J. Agric. and Food Chem.,* **51:** 987-991.

Oka, Y.; Cohen, Y. and Spiegel, Y. 1999. Local and systemic induced resistance to the root-knot nematode in tomato by DL.β-amino-n-butyric acid. *Phytopathology.* **89:** 1138-1143.

Paris-Palacios, S.; Biagianti-Risbourg, S. and Vernet, G. 1998. Hepatic disorders induced by a sublethal concentration of procymidone (fungicide dicarboximide) in Brachydanio rerio (Teleostei, Cyprinidae): histo-cytological approach. *Bull. Fr. Peche. Piscic.,* **350:** 547–562.

Paxton, J.D. 1981. Phytoalexins: A working redefinition. *Phytopathol. Z.* **101**: 106-109.

Stevenson, W.R. 1993. Management of early and late blight. pp141-147. In: Randall, C. Rove (ed.). *Potato Health Management.* APS Press, St., Paul MN 178 pp.

Stoessl, A. 1982. Biosynthesis of phytoalexins. Pages: 133-180. In: *Phytoalexins.* Bailey J.A. and J.W. Manfield (eds.). Glasgow and London, UK, Blackie.

Stoessl, A.; Stothers, J.B. and Ward, E.W.B. 1978. Sesquiterpenoid stress compounds of the Solanaceae. *Phytochemistry,* **15**: 855-872.

Suijkowski, L.S.; Fry, B.A.; Power, R.J.; Goodwin, S.B. ; Reever, R.A. Hamlen, T.L. and Fry, W.E. 1995. Sensitivities of Mexican isolates of *Phytophthora infestans* to chlorothalonil, cymoxanil and metalaxyl. *Plant Dis.,* **79:** 1117-1120.

Suijkowski, L.S.; Goodwin, S.B. and Fry, W.E. 1996. Changes in specific virulence in Polish populations of *Phytophthora infestans.* 1985-1991. *Europ. J. Plant Pathol.,* **102**: 555-561.

Szafrauek, B.; Chrapkwskak, C.; Pawinska, A. and Szafrauek, A. 2005. Analysis of leaf surface sesquiterpenes in potato varieties. *J. Agric. and Food Chem.,* **53**: 2817-2822.

Van Wees, S.C.M.; De Swart, E.A.M.; Van Plek, J.A. ;VanLoon, L.C. and Pieterse, C.M.J. 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate and jasmonate dependent pathways in *Arabidiopsis thaliana*. Proc. Nat. Acad. Sci., USA, **97**:8711-8716.

Vernooij, B.; Friedrich, L.; Goy, P.A.; Staub, T.; Kessmann, H. and Ryals, J. 1995. 2,6-dichloroisonicotinic acid induced resistance to pathogens without the accumulation of salicylic acid. *Mol. Plant Microbe Infestations*, **8**: 228-234.

Wastie, R.L.; Stewart H.E.; Mackay, G.R.; Bevan, J. and Lennartson, M. 1993. Exploiting blight resistance for sustainable potato cultivation. *Biologi Italiano*, **22**: 18-22.

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إحداث المقاومة في نباتات البطاطس ضد مرض الندوة المتأخرة وعلاقته بتحفيز تكوين الفيتوالكسينات

مصطفى حلمي مصطفى- عماد الدين على مصطفى جادو
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يعد مرض الندوة المتأخرة في البطاطس المتسبب عن *Phytophthora infestans* أحد أخطر الأمراض التي تصيب البطاطس في جميع أنحاء العالم وفي بعض المناطق في مصر. اختبرت بعض المستخلصات الطبيعية (محلول لبن جوز الهند (٢%) ومستخلص نورات المانجو المشوهة (٧,٥%) و السينكوسين (٠,١%) والأجريسبون (٠,١%) والمركبات الكيماوية (ايثايل حمض ساليسليك (٠,١٢٥/ لتر) وحمض الجاسمونيك (٠,١٢٥/ لتر) على ابتعاث المقاومة ضد هذا المرض. وقد اختيرت هذه المواد على أساس أنها تؤدي لإحداث زيادة في نشاط إنزيم البيروكسيداز في الدرنات المعاملة، تم رش المحاليل المائية المختبرة على نباتات البطاطس صنف دياموند المنزرع تحت ظروف الحقل عندما وصلت النباتات لعمر ٦٠ يوما وكل عشرة أيام.

تم تقدير الإصابة بالندوة المتأخرة عند بداية ظهور الأعراض، حيث تم تقدير متوسط عدد البقع/ نبات ومتوسط قطر البقعة/ نبات، تم تقدير شدة الإصابة النهائية بعد ٣ أسابيع من ظهور المرض.

أوضحت النتائج أن محلول لبن جوز الهند ومستخلص نورات المانجو المشوهة و ايثايل حمض ساليسليك والسينكوسين والأجريسبون قد أدوا لاختزال عدد البقع/ نبات مقارنة بالنباتات غير المعاملة. وفقد وجد أن كل المركبات المختبرة قد اختزلت قطر البقعة وكانت أفضل المواد هي ايثايل حمض الساليسيليك، حيث أختزل قطر البقعة من ١١,٢±١,٠٥ إلى ٢,٧±٢,١٢ ملليمتر.

أدى استخدام حمض الجاسمونيك ومستخلص نورات المانجو المشوهة الى نقص شدة الإصابة النهائية للمرض في النباتات المعاملة، حيث اختزلت شدة الإصابة من ٥٠% على النباتات غير المعاملة إلى ١% على النباتات المعاملة.
أثبتت النتائج أيضا أن جميع المواد المختبرة تستحث تكوين الفيتوالكسينات ، إلا أن عددها تباين من محلول لآخر. ففي الوقت الذي أحدث فيه حمض الجاسمونيك بناء خمسة مركبات فأن مستخلص نورات المانجو المشوهة أدى إلى بناء مركبين.

بحساب معامل الارتباط بين عدد المركبات المبتعثه وشدة الإصابة وجد أنها بلغت -٠,٦٤ مما يؤكد على أن زيادة عدد الفيتوالكسينات المنبعثة يؤدي لانخفاض شدة الإصابة على النباتات.