OPEN ACCESS Int. J. Phytopathol. 07 (02) 2018. 53-61 DOI: 10.33687/phytopath.007.02.2383

Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

ELICITATION OF PHYTOALEXIN RISHITIN IN POTATO TUBER SLICES INFECTED BY *FUSARIUM* SPP., DOES IT CONSIDER A FACTOR OF PATHOGENICITY?

Mostafa H. Mostafa*
Plant Pathology Department, Faculty of Agriculture, Ain Shams University, 11241 Cairo, Egypt.

**Corresponding Author Email: mostafa_mohamed@agr.asu.edu.eg*

A B S T R A C T

Potato tubers, under storage conditions, suffer from Fusarium dry rot disease caused by *Fusarium* spp. leading to devastating losses of stored tubers. Newly harvested tubers are resistance toward *Fusarium* infection, but disease becomes more vigorous by increasing storage period. Inoculation of potato tuber slices of Desiree *cv.* (highly susceptible) or Spunta *cv.* (highly resistant) either by a weak pathogen (*F. moniliforme*) or severe pathogen (*F. solani*) and tracking accumulation of phytoalexin rishitin in inoculated tissues at different intervals from inoculation revealed that Desiree *cv.* accumulated high amounts of rishitin in a very short time in comparison to another three tested situations. Dipping of potato tuber slices of both *cvs.* in different concentrations of the known antibiotic chloramphenicol prior to inoculation with a weak pathogen (*F. moniliforme*) led to increased disease severity associated with accumulation huge amounts of rishitin in inoculated tissues. The correlation coefficient between disease severity and rishitin production was +0.83. Moreover, the reaction of spunta *cv.* toward inoculation by a weak pathogen was changed from the state of resistance to very severe susceptibility. Since chloramphenicol affects protein synthesis on 70S ribosome's, it was postulated that it prevents synthesis of factor(s) take a part in cell death during infection. Mitochondria extracted from potato tuber tissues secret compound(s) decreased rishitin synthesis in potato tuber slices treated with mycelia extract of *F. solani*. Treatment of isolated mitochondria with this antibiotic led to the elimination of this factor(s) from their secretions. Results obtained revealed that the elicitation of phytoalexins may consider one of pathogenicity factor of such system.

Keywords: *Fusarium moniliforme, Fusarium solani,* Spunta *cv.*, Desiree *cv.*, Chloramphenicol, mitochondria, dry rot, phytoalexin.

INTRODUCTION

Fusarium dry rot is one of the most important diseases of potato (*Solanum tuberosum* L.), affecting tubers in storage and seed pieces after planting. Fusarium dry rot of seed tubers can reduce crop establishment by killing developing potato sprouts. The response of potato tuber cultivars to *Fusarium* infection could be dividing into resistant *cvs*. and susceptible *cvs*. (Leach, 1981; Mejdoub *et al.*, 2015). The molecular factors associated with the state of resistance were subject of research from different points of view. The role of steroid glycoalkaloids (SGA), phenol and related enzymes and their polymer lignin in relation to cultivar resistance and rapid suberization of wounded tubers were postulated as actors of resistance (Allen and Kuc, 1964; Corsini and Pavek, 1980; Ashour and Mostafa, 1983; Lulai and Corsini, 1998; McCue, 2009). On the other hand, the main factors intensively investigated in relation to susceptibility were the cell wall hydrolyzing enzymes (Cooper *et al*., 1981, Ashour and Mostafa, 1983) and fusaric acid production (Venter and Steyn, 1998) by invading pathogen as a factor of pathogenicity.

Phytoalexins (sesquiterpenoid stress metabolites) were found to accumulate in potato tuber tissues infected by *Fusarium* spp. in both cases of interactions; resistance or susceptibility. (Stoessl *et al.*, 1976; Corsini *et al.*, 1977; Corsini and Pavek, 1980). For many years much of the research in the reason of resistance has focused on the phytoalexins production in inoculated tissues, especially rishitin and lubimin. If these compounds consider disease resistance factors in potato tubers, therefore, successful pathogens may have developed mechanisms for

tolerating them. In this respect (Desjardins *et al.*, 1989; Desjardins *et al.*, 1992) have found that *Gibberella pulicaris* (*F. sambucinum*) can detoxify potato phytoalexins and the ability of field strains of this fungus to cause dry-rot is correlated with their ability to detoxify phytoalexins produced by potato tubers.

The aim of the present study is to answer a very important question: Do the causal agents of potato dry rot elicit phytoalexins in infected tissue accidentally or they produce them intentionally as a factor of pathogenicity? In order to answer this question, the following steps were carried out: i) tracking rishitin production either in resistant or susceptible *cvs*. inoculated either by severe pathogen i. e. *Fusarium solani or* by a weak pathogen *Fusarium moniliforme*; ii) enhancement of SSM production in inoculated slices by chloramphenicol treatment (Ersek *et al.*, 1973) and study the effect of such treatment on disease severity due to inoculation with weak pathogen; and finally propose how chloramphenicol enhance phytoalexin production in inoculated tissues although it does not act as a phytoalexin elicitor. *Fusarium solani* (Martins) Appl & Wollenweber and *Fusarium moniliform* Sheld, were isolated from potato tubers showed dry rot symptoms. *F. solani* was found to be a very severe pathogen and *F. moniliforme* was a very weak pathogen. Desire *cv.* Is highly susceptible to Fusarium infection and Spunta was resistant to both fungi (El-Hassan *et al.*, 2007).

MATERIALS AND METHODS

Potato tubers: Potato tubers of *cvs*. Desiree and Spunta were obtained from Institute of Vegetable Research, Agricultural Research Centre, Giza, Egypt. The tubers were stored at least for one month at room temperature.

Fungi: *Fusarium solani* and *F. moniliforme* were cultured on PDA medium. A spore suspension in sterilized distilled water was prepared from 10 days old cultures, and spore density was adjusted before use to be 10^6 spores/ml.

Preparation of potato tuber slices, chloramphenicol treatment and inoculation. Tubers were washed several times with tap water, dried, then surface sterilized in 5% $NaCl_3$ for 5 minutes. After washing in sterilized distilled water, the tubers were cut into 1 cm-thick slices with the exclusion of the top and the basal parts. Slices were washed in sterilized distilled water then dipped in chloramphenicol suspension for 2 hours. The antibiotic was tested in 6 concentrations (0, 50, 100, 200, 400 and 800 ppm). Slices were transferred to Petri dishes (15 cm in diameter) containing tow wetted filter papers. Five slices were put in every dish and three dishes for each particular treatment were used. Inoculation of slices was carried out one hour after they had been put in dishes by pouring gently 0.5 ml of spore suspension over every slice. Dishes were incubated at 24±1 °C in the dark for 3 days.

Disease severity: Three days after inoculation, each slice was divided into two symmetrical portions; one to determine disease severity and the other to extract phytoalexins. For determination of disease severity, the upper inoculated surface was removed then ground in 10 ml distilled water. A number of conidiospores were counted using haemocytometer on a light microscope. Disease severity was expressed as a number of spores/ cm^2 of the inoculated surface.

Determination of rishitin in inoculated potato tuber slices or treated by mycelia extract of *F. solani*.

A. Preparation of mycelia extract of *Fusarium solani*: The fungus was grown in an autoclaved solution of Richard's medium for 15 days at 25±1 °C. Fungal mats were homogenized in distilled water (1 gm fresh weight/ 10 ml). Homogenates were filtered through two layers of cheesecloth's, then autoclaved at 1 kg/cm^2 for 15 min. Total sugars (as glucose) was determined by anthrone method (Dische, 1962). Autoclaved mycelia extract was adjusted to be 100 µg sugars/ ml distilled water before using.

B. Inoculation of slices by *F. solani* or treatment with mycelia extract: Slices of Desiree *cv.* were prepared and treated with chloramphenicol as mentioned above, then treated by mycelia extract (0.5 ml/ slice) or inoculated with *F. solani*. After 72 h rishitin content was determined in inoculated slices or stressed by mycelia extract and its content was expressed as µg / cm^2 of inoculated or treated by mycelia extract slice surface. It could be mentioned that slices inoculated by *F. solani* were incubated at 24±1 °C, meanwhile, slices treated with mycelia extract were incubated at 21±1 °C. This experiment was carried out twice.

Quantitative determination of rishitin in inoculated slices or stressed by mycelia extract: Rishitin (the main SSM) produced by both *cvs*. i.e. Desiree or Spunta was isolated, purified using thin layer chromatography according to the method described by (Alves *et al.*, 1979) to identify the main phytoalexin in tested cultivars. Methanolic solutions containing SSMs were spotted on silica gel/TLC-cards 0.2mm plates (Fluka) which were developed to a height of 155 mm in an

ascending solvent system of ethyl acetate-cyclohexane (1:1, v/v). Plates were stained by spraying with chloroform saturated with antimony. Quantitative determination of rishitin was carried out according to the method adopted by Heisler *et al*. (1978) and it was expressed as µg / cm^2 of the inoculated surface.

Time-course of rishitin production in inoculated slices: Slices of both Desiree and Spunta *cvs*. were prepared and inoculated either by *F. solani* or *F. moniliforme* as mentioned above and incubated at 24±1°C. After 24, 48 and 72 h from incubation, slices were taken and the upper inoculated surface (3 mm) was removed for quantitative determination of rishitin. Data were expressed as µg/ cm^2 of the inoculated surface.

Effect of chloramphenicol on the growth of *F. solani* and *F. moniliforme*, sporulation, spore germination and germ tube length: Linear growth method was carried out on PDA containing the particular amount of the antibiotic. Sporulation was determined on the same plate at 12TH cultural old. Spore suspension from non-chloramphenicol treated plates was prepared then mixed with appropriate amount of the antibiotic. Twenty drops from each particular treatment were distributed on 10 glass slides. Percentage of spore germination and germ tube length were measured after 24h from incubation at 25±1°C. (Data obtained indicated that the antibiotic had no effect on all determined phenomena. It does not cause any deleterious effect on fungal growth, spore germination and germ tube length.

Isolation of mitochondria and preparation of mitochondrial secretions (MS): Mitochondria from 880 gm tissues of potato tubers were isolated using the method described by Laval-Martin and Mazliak (1979) bovine serum albumin was not added during mitochondrial extraction. For the preparation of MS, mitochondrial suspension (100 ml) was incubated at 20±1°C for 16 h then centrifuged at 12000g for 35 min. The supernatant (MS) was then separated. Sediment mitochondria were washed in distilled water, sediment again then weighed.

In a separate experiment, the mitochondrial suspension was prepared (100 ml) then divided into two equal portions. In one portion, 10 mg of chloramphenicol was added to give a final concentration of 200 ppm. Mitochondrial chloramphenicol suspension was kept for 16 h at 20±1 °C then centrifuged for sedimentation of mitochondria as mentioned above, centrifugation was carried out at 4 °C using Beckman centrifuge model J2 21.

A number of experiments and statistical analysis: All previously described experiments were carried out three times unless otherwise states in context, they repeated twice. Standard deviation (δ) was calculated according to (Ghahramani, 2000). The correlation coefficient between disease severity and rishitin content and student t-test were calculated according to (Kenney and Keeping, 1951).

RESULTS

Time-course of rishitin accumulation in inoculated potato tuber slices: Desiree and Spunta *cvs*. produced rishitin as the main phytoalexin due to their inoculation by *Fusarium* spp. Rishitin content was determined in inoculated potato tuber slices either by *F. solani* or *F. moniliforme* after 24, 48 and 72 h from inoculation. Data illustrated in table 1 indicate that rishitin rapidly induced in Desire *cv.* inoculated by sever pathogen *F. solani*, reached its maximum 48 h after inoculation.

Table 1. Time-course of rishitin induction in Desiree *cv.* inoculated with either by a severe pathogen (*F. solani*) or weak pathogen (*F. moniliforme*)

Time after inoculation	µg rishitin/ cm² of inoculated surface	
	F. solani	*F. moniliforme*
24 hr	75 ± 8.5	30 ± 3.7
48 hr	112 ± 12.2	55 ± 6.2
72 hr	120 ± 15.0	50 ± 7.3

On the other hand, rishitin induction in slices inoculated with the weak pathogen *F. moniliforme* was lower to a great extent than that found in slices inoculated with severe pathogen i.e. *F. solani*. Rishitin production in slices of Spunta *cv.* (more resistance) inoculated either by both pathogens was un-detectable 24 h after inoculation then it started to increase (table 2). As indicated in table 1 and table 2 rishitin induction in highly susceptible *cv.* Desiree was very high due to inoculation with severe pathogen in comparison to the amount of rishitin elicited in resistant *cv*. Spunta.

Table 2. Time- course of rishitin induction in Spunta *cv.* inoculate either with by severe pathogen (*F. solani*) or weak pathogen (*F. moniliforme*)

Time after inoculation	μg rishitin/ cm² of inoculated surface	
	F. solani	*F. moniliforme*
24 hr	0	0
48 hr	57 ± 13.0	10 ± 2.3
72 hr	88 ± 15.0	60 ± 8.4

Effect of chloramphenicol treatment of potato tuber slices on disease severity and rishitin production due to inoculation with *F. moniliforme*: Disease severity on chloramphenicol treated potato tuber slices Desiree *cv.* or Spunta *cv.* inoculated with a weak pathogen *F. moniliforme* either treated or not was determined after 72 h from inoculation. Data from this study are presented in tables (3 and 4). The increment of disease severity was increased by increasing chloramphenicol concentration to 200 ppm then slightly decreased. In such tissues, rishitin accumulation was also increased due to infection. In case of Spunta. (resistant *cv.*) inoculated by the weak pathogen *F. moniliforme,* it was noticed that the state of interaction was changed from the state of resistance (in control slices) to the state of susceptibility and rishitin content was also increased in rotted tissues. Calculation correlation coefficient between disease severities and elicitation of rishitin reached + 0.83.

Table 3. Effect of dipping of potato tuber slices (Desiree *cv.*) in chloramphenicol (ppm) on disease severity and rishitin production in potato tuber tissues inoculated with *F. moniliforme.*

Chloramphenicol (ppm)	No. of spores/cm² of inoculated surface (X10²)	Rishitin μg/ cm² of inoculated surface
0	10 ± 2.0	40 ± 8.4
50	135 ± 10.4	60 ± 5.2
100	165 ± 15.2	105 ± 10.3
200	170 ± 13.4	140 ± 12.8
400	170 ± 12.2	210 ± 18.4
800	160 ± 18.1	200 ± 20.2

Data was determined 72 hr after inoculation.

Table 4. Effect of dipping of potato tuber slices (Spunta *cv.*) in chloramphenicol (ppm) on disease severity and rishitin production due to inoculation with *F. moniliforme*.

Chloramphenicol (ppm)	No. of spores/cm² of inoculated surface (X10²)	Rishitin μg/ cm² of inoculated surface
0	0	30 ± 5.0
50	20 ± 3.8	60 ± 6.5
100	30 ± 4.8	150 ± 12.2
200	210 ± 15.2	190 ± 17.3
400	160 ± 12.4	190 ± 20.2
800	144 ± 12.2	200 ± 20.4

Data was determined 72 hr after inoculation.

A comparison study between the ability of potato tuber slices for induction of rishitin due to inoculation by *F. solani* or treated with its mycelia extract under the influence of chloramphenicol treatment: Rishitin accumulation in potato tuber slices previously treated with different concentrations of chloramphenicol inoculated by spore suspension of *F. solani* or treated with autoclaved mycelia extract of was determined 72 h after incubation. Data are presented in (table 5 and Figure 1). Incubation was carried out at 25±1°C for slices inoculated by spore suspension or at 20±1°C for slices treated by mycelia extract. Rishitin was determined after 72 h from incubation.

Table 5. Rishitin production (μg/cm^2) in inoculated slices of potato tuber tissues inoculated by spore suspension of *Fusarium solani* or its mycelia extract under the effect of chloramphenicol treatment.

Chloramphenicol (ppm)	*F. solani* inoculation		Mycelia extract of *F. solani*		Control
	Exp. I	Exp. II	Exp. I	Exp. II	
0	42.8±10.9	73.6±11.5	255±27	236±32	Non detectable
50	77.7±24.2	117±30.3	360±36	380±47.2	“
100	133.4±32.6	156.5±38.4	492±42	483±25.3	“
200	240.5±34.9	268.5±34.3	600±58	632±37.2	“
400	204.8±34.9	220.8±40.7	582±38	620±46.8	“
800	205.9±43.6	204.2±41.7	570±29	580±41.2	“

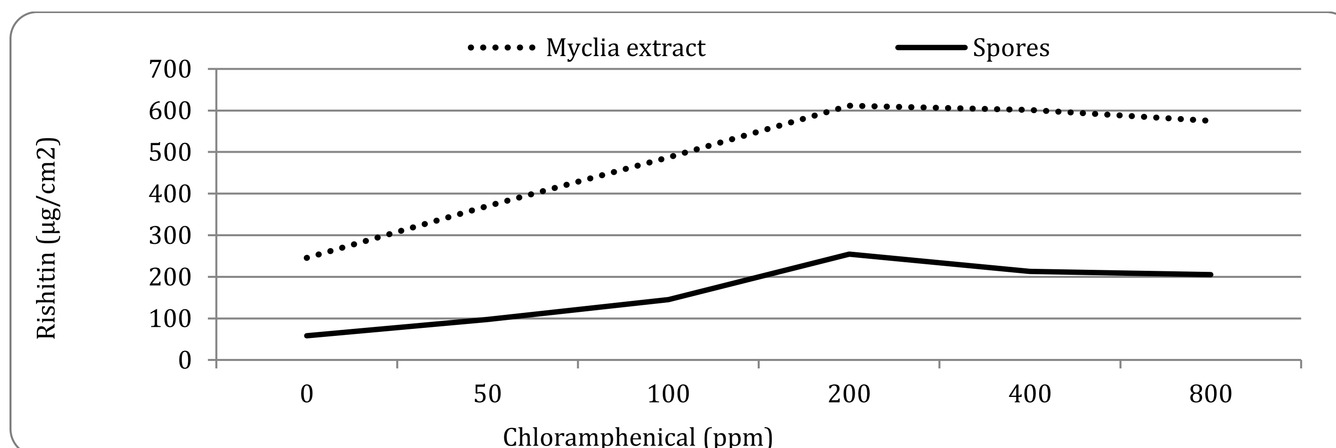

Figure 1. Rishitin production (μg/ml^2) of inoculated slices of potato tuber tissues inoculated with a spore suspension of *Fusarium solani* or its mycelia extract under the effect of chloramphenicol treatment.

Incubation was carried out at 25±1°C for slices inoculated by spore suspension or at 20±1 °C for slices treated by mycelia extract. Rishitin was determined after 72 h from incubation. (Data are average of two distinct experiments). As shown in Figure 1, *F. solani* and its mycelia extract have the ability to elicited rishitin induction in potato tuber tissues. The amount of rishitin produced in tissues treated with mycelia extract exert that induced due to inoculation by *F. solani* spore suspension. This may be attributed to temperature degree of incubation of treated or inoculated slices.

Effect of mitochondrial secretions on rishitin production by potato tuber slices treated by mycelia extract of *F. solani*: Potato tuber slices were treated by secretions of mitochondria isolated from newly harvested potato tubers prior to treatment with mycelia extract, then rishitin was determined in treated slices. Data tabulated in table (6) indicate clearly that accumulated rishitin in potato tuber slices significantly reduced by 61% in comparison to control none treated by mitochondrial secretions. In addition, incubation of mitochondrial suspension for 16 h in chloramphenicol suspension (200 ppm) then centrifuged for the elimination of mitochondrion and chloramphenicol, their secretions were also tested for their effect on rishitin production in potato tuber slices treated with mycelia extract.

Table 6. Effect of treatment of potato tuber slices with mitochondrial secretions on rishitin production (μg/ cm^2 of the treated surface) due to treatment with mycelia extract of *Fusarium solani* and the effect of chloramphenicol on these secretions.

Treatment	Experiment I		Experiment II	
	μg rishitin/cm^2 of treated surface	t. calculated	μg rishitin/cm^2 of treated surface	t. calculated
A	228±49		269±79	
B	140±33	4.64	128±43	4.15
C	286±48		248±63	
D	239±63		243±70	

t. theoretical at 95%= 2.1, t. theoretical at 99%=2.9

Data from this study are presented also in table 6. As indicated from this data, chloramphenicol eliminates factor(s) caused reduction of rishitin synthesis in potato tuber tissues.

A: Control (A): Potato tuber slices treated with mycelia extract in the buffer solution used for isolation of mitochondrion. B: Potato tuber slices treated with mitochondrial secretions then treated with mycelia extract. C: Control (B): Potato tuber slices treated with mycelia extract mixed with chloramphenicol then centrifuged for the elimination of the antibiotic then treated by mycelia extract D: Potato tuber slices treated with secretions of mitochondrion treated with chloramphenicol then centrifuged for the elimination of mitochondrion and antibiotic.

DISCUSSION

Due to the exposure of plants to microorganisms, plant accumulates low molecular weight compounds called phytoalexins (Roop Singh and Kuldeep Sing Chandrawat, 2017). Solanaceous plants produce sesquiterpenoid stress metabolites due to infection by incompatible races of fungi, Oomycetes, bacteria and viruses (Paxton, 1981; Allen and Kuc, 1964). There is a lot of evidence demonstrated that phytoalexins may play a major role in plant disease resistance against pathogen attacks (Ingham, 1972; Allen and Kuc, 1964; Stoessl *et al.*, 1976; Darvill and Albersheim, 1984). However, in some compatible interactions (state of susceptibility), it has been observed that host accumulates high levels of phytoalexins associate with infection, i.e. bean (*Phaseolus vulgaris*) infected with *Fusarium solani* f.sp. *phaseoli* (Kistler and VanEtten, 1981); peas (*Pisum sativum*) infected with *F. solani* f.sp *pisi* (VanEtten, 1982); potato (*Solanum tuberosum*) infected with Fusarium tuber rot (Stoessl *et al.*, 1976).

(VanEtten, 1982) in his study on the differences between virulent and avirulent isolates of *F. solani* f.sp. *pisi* revealed that isolates demethylate (detoxify) the phytoalexin pisatin effectively infected pea plants but those which were unable to detoxify pisatin were avirulent. In the same direction (Desjardins *et al.*, 1992) have found that the fungus *Gibberella pulicaris (F. sambucinum*) detoxify sesquiterpene phytoalexins (rishitin and lubimin) accumulate during infection of potato tuber tissues and they found that the virulence of this fungus depends upon its ability to detoxify these phytoalexins.

Concerning Fusarium- potato dry rot, we noticed that the newly harvested potato tubers –in general- are resistant to *Fusarium* spp. Infection. It is also well established that the ability of such tubers to produce sesquiterpene stress metabolites (phytoalexin) is very restricted (Bostock, 1983). The ability of *Fusarium* spp. to infect such tubers increases by increasing of storage period. Herein, we postulated that phytoalexin production in inoculated potato tuber tissues may consider the factor of pathogenicity in such system (*Fusarium*- potato tuber).

To test such hypothesis, the present work passed through two steps. The first was to determine the time-course of accumulation of phytoalexin rishitin at different intervals from inoculation of potato tuber slices of Desiree (susceptible) or Spunta (resistance) *cvs.* either inoculated by an aggressive pathogen (*Fusarium solani*) or weak pathogen (*F. moniliforme*). The second step entailed treatment of potato tuber slices of both *cvs.* with different concentrations of the known antibiotic chloramphenicol before they had been inoculated with the weak pathogen *F. moniliforme* then the determination of disease severity and rishitin accumulating in inoculated tissues. Also, the effect of chloramphenicol on fungal growth, sporulation, spore germination and germ tube length were investigated.

At the first step, a sharp increase in rishitin was pronounced in potato tuber slices Desiree *cv.* (highly susceptible) 24 h after inoculation by severe pathogen relative to the three other situations (susceptible *cv.* inoculated by a weak pathogen and resistant *cv.* inoculated either by both pathogens).

Concerning the second step, slices of both *cvs*. were treated with different concentrations of chloramphenicol before they had been inoculated by a weak pathogen *F. moniliforme.* Data showed that disease severity sharply increased by increasing chloramphenicol concentration accompanied by accumulation of high levels of phytoalexin rishitin. Moreover, spunta *cv.* (resistant) inoculated by a weak pathogen gave very interesting phenomenon whereas, the interaction between the weak pathogen and the resistant *cv.* completely changed from state of resistance to very severe infection accompanied by accumulation of high amount of rishitin. The correlation coefficient between disease severity and the amount of accumulated rishitin reached +0,82. This high value of correlation indicates the presence of high correlation between disease severity and synthesis of phytoalexin.

In the work conducted by (Ersek *et al.*, 1973), in which

they studied the effect of treatment of potato tuber slices by chloramphenicol on interrelationship between compatible races of *Phytophthora infestans* and potato tuber slices, they discover that the interaction between *P. infestans* and potato tuber was changed from compatible situation to incompatible situation which was accompanied by accumulation of phytoalexin rishitin, the factor of resistance in this system. In the present study, chloramphenicol led to change the interaction between Fusarium and potato tuber tissues from the state of resistance to the state of susceptibility accompanied by accumulation of high levels of rishitin. How could one explain the causal of such results? It could simply say that the factor responsible for resistance of potato tubers to *P. infestans* is itself responsible for disease severity in the case of Fusarium tuber rot. Some evidence from genomic studies revealed that gene of susceptibility in barley *HvRAC1* confers resistance of the same plant against *Magnaporthe oryzea* (Pathuri *et al.*, 2008).

Accumulation of phytoalexin rishitin in this system (potato tuber- *Fusarium*) may have a considerable role in one or more of the following statement: i) prevention of steroid glycoalkaloids (SGA) induction (Allen and Kuc, 1964). In this respect, some authors believe that SGA accumulation considers a factor of resistance of potato tubers to *Fusarium* infection (Allen and Kuc, 1964). On the other hand, (McKee, 1959) doubted the role of SGS in resistance of potato tubers to Fusarium dry rot. He found that the toxicity of SGA to *Fusarium* spp. depends on pH, and the growth of the fungus is not sensitive to SGA. Moreover, the time required for SGA deposition in a toxic level is too long. As a matter of fact, it is difficult to neglect the role of SGA in limitation of Fusarium spp. Infection. Ii) Exhaust of ATP in phytoalexin production by infected tissues, and this may alter the cells for production of an energy-dependent resistant factor(s). iii) Alter membrane permeability of plant cells due to the accumulation of phytoalexin (Lyon, 1980) leading to uncontrollable physiological processes.

From the obtained results a very important question was raised: how chloramphenicol enhanced accumulation of phytoalexin rishitin in treated inoculated tissues, although it does not act as a phytoalexin elicitor? It is well established that this antibiotic inhibits protein synthesis on 70S ribosomes (Kroon, 1965; Coutsogeorgopoulos, 1966; Yang and Scandalios, 1977), accordingly it could postulate that mitochondria synthesize a factor(s) which may affect phytoalexin induction either by inoculated slices or treated by the fungal mycelial extract. Therefore, mitochondrial secretions of potato tuber tissues were tested on rishitin production by potato tuber tissues treated with *F. solani* mycelia extract. Data obtained confirmed our hypothesis that mitochondria synthesize factor(s) control rishitin production by potato tuber tissues. How do mitochondria control this process? It is well established that phytoalexin synthesis requires living cells and dead cells have not the ability to synthesize phytoalexins. Since *Fusarium* spp. Consider hemibiotrophs i.e. require living cells in the early stage of infection then plant cells undergo death due to infection, thereafter, the amount of phytoalexin accumulate during infection will greatly depend upon the velocity of cell death. In this respect (Suzuki *et al.*, 1999) in their work titled mitochondrial regulation of cell death: mitochondria are essential for procaspase 3-p21 complex formation to resist Fas-mediated cell death, they concluded that mitochondria are necessary for procaspase-3-p21 complex formation and proposed that mitochondrial role during cell death is not only death induction but also death suppression. Moreover, (Li *et al.*, 2005) revealed that chloramphenicol-induced mitochondrial stress increases p21 expression and prevent cell apoptosis through a p21-dependent pathway.

REFERENCES

Allen, E. and J. Kuc. 1964. Steroid alkaloids in disease resistance of white potato tubers Phytopathology. American Phytopathological Society 3340 Pilot Knob Road, ST Paul, MN 55121.

Alves, L. M., E. G. Heisler, J. C. Kissinger, J. M. Patterson and E. B. Kalan. 1979. Effects of controlled atmospheres on the production of sesquiterpenoid stress metabolites by white potato tuber: Possible involvement of cyanide-resistant respiration. Plant Physiology, 63: 359-62.

Ashour, W. and M. Mostafa. 1983. Studies on the interaction between potato tuber tissues and Fusarium solani. I. Phenylalanine ammonia lyase, orthodihydroxy phenol oxidase, soluble peroxidase and cell wall hydrolyzing enzymes activities. Annals of agricultural science.

Bostock, R. M. 1983. Effects of Potato Tuber Age and Storage on Sesquiterpenoid Stress Metabolite Accumulation, Steroid Glycoalkaloid Accumulation, and Response to Abscisic and Arachidonic Acids. Phytopathology, 73: 435.

Corsini, D., J. Pavek and G. Fenwick. 1977.

Characterization of Fusarium dry rot resistance in potato breeding clones. Proceedings American Phytopathological Society.

Corsini, D. L. and J. J. Pavek. 1980. Phenylalanine ammonia lyase activity and fungitoxic metabolites produced by potato cultivars in response to Fusarium tuber rot. Physiological Plant Pathology, 16: 63-72.

Coutsogeorgopoulos, C. 1966. On the mechanism of action of chloramphenicol in protein synthesis. Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis, 129: 214-17.

Darvill, A. G. and P. Albersheim. 1984. Phytoalexins and their Elicitors-A Defense Against Microbial Infection in Plants. Annual Review of Plant Physiology, 35: 243-75.

Desjardins, A. E., H. W. Gardner and R. D. Plattner. 1989. Detoxification of the potato phytoalexin lubimin by Gibberella pulicaris. Phytochemistry, 28: 431-37.

Desjardins, A. E., H. W. Gardner and K.-M. Weltring. 1992. Detoxification of sesquiterpene phytoalexins byGibberella pulicaris (Fusarium sambucinum) and its importance for virulence on potato tubers. Journal of Industrial Microbiology, 9: 201-11.

Dische, Z. 1962. Colour reactions of carbohydrates. Methods in carbohydrate chemistry, 1: 477-512.

El-Hassan, K., M. El-Saman, A. Mosa and M. Mostafa. 2007. Variation among Fusarium spp. the causal of potato tuber dry rot in their pathogenicity and mycotoxins production. Egyptian Journal of Phytopathology, 35: 53-68.

Ersek, T., B. Barna and Z. Kiraly. 1973. Hypersensitivity and the resistance of potato tuber tissues to Phytophthora infestans. Acta phytopathologica.

Ghahramani, S. 2000. Fundamentals of probability Prentice Hall New Jersey:.

Ingham, J. L. 1972. Phytoalexins and other natural products as factors in plant disease resistance. The Botanical Review, 38: 343-424.

Kenney, F. and E. Keeping. 1951. Mathematics of statistics-part two.

Kistler, H. C. and H. D. VanEtten. 1981. Phaseollin metabolism and tolerance in *Fusarium solani* f. sp. *phaseoli*. Physiological Plant Pathology, 19: 257-71.

Kroon, A. M. 1965. Protein synthesis in mitochondria III. On the effects of inhibitors on the incorporation of amino acids into protein by intact mitochondria and digitonin fractions. Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis, 108: 275-84.

Laval-Martin, D. and P. Mazliak. 1979. Plant physiology, practical works and planned works.

Leach, S. S. 1981. Resistance of selected potato cultivars and clones to fusarium dry rot. Phytopathology, 71: 623.

Li, C.-H., S.-L. Tzeng, Y.-W. Cheng and J.-J. Kang. 2005. Chloramphenicol-induced Mitochondrial Stress Increases p21 Expression and Prevents Cell Apoptosis through a p21-dependent Pathway. Journal of Biological Chemistry, 280: 26193-99.

Lulai, E. C. and D. L. Corsini. 1998. Differential deposition of suberin phenolic and aliphatic domains and their roles in resistance to infection during potato tuber (Solanum tuberosumL.) wound-healing. Physiological and Molecular Plant Pathology, 53: 209-22.

Lyon, G. D. 1980. Evidence that the toxic effect of rishitin may be due to membrane damage. Journal of Experimental Botany, 31: 957-66.

McCue, K. F. 2009. Potato glycoalkaloids, past present and future. Fruit, Vegetable and Cereal Science and Biotechnology, 3.

McKee, R. K. 1959. Factors affecting the toxicity of solanine and related alkaloids to *Fusarium caeruleum*. Journal of General Microbiology, 20: 686-96.

Mejdoub, T. B., H. J. Khiareddine and M. D. Remadi. 2015. Interactions between four *Fusarium s*pecies in potato tubers and consequences for fungal development and susceptibility assessment of five potato cultivars under different storage temperature. Journal of Plant Pathology and Microbiology, 06: 1-12.

Pathuri, I. P., N. Zellerhoff, U. Schaffrath, G. Hensel, J. Kumlehn, K.-H. Kogel, R. Eichmann and R. Hückelhoven. 2008. Constitutively activated barley ROPs modulate epidermal cell size, defense reactions and interactions with fungal leaf pathogens. Plant Cell Reports, 27: 1877-87.

Paxton, J. D. 1981. Phytoalexins-A working redefinition. Journal of Phytopathology, 101: 106-09.

Stoessl, A., J. B. Stothers and E. W. B. Ward. 1976. Sesquiterpenoid stress compounds of the Solanaceae. Phytochemistry, 15: 855-72.

Suzuki, A., Y. Tsutomi, N. Yamamoto, T. Shibutani and K. Akahane. 1999. Mitochondrial regulation of cell death: Mitochondria are essential for procaspase 3-

p21 complex formation to resist Fas-mediated cell death. Molecular and Cellular Biology, 19: 3842-47.

VanEtten, H. 1982. Phytoalexin detoxification by monooxygenases and its importance for pathogenicity. Plant Infection: The Physiological and Biochemical Basis.

Yang, N. S. and J. G. Scandalios. 1977. Effects of cycloheximide and chloramphenicol on the synthesis of polypeptides found in three subcellular fractions of maize scutellum. Plant Physiology, 59: 1067-71.

Publisher's note: EScience Press remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018.