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Solanapyrones Produced by Turkish Isolates of *Ascochyta rabiei* and Their Phytotoxicity on Chickpeas

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ABSTRACT

Randomly selected four isolates were grown on Czapek Dox liquid medium supplemented with metal cations for 7, 14 and 21 days in order to determine kinetics of solanapyrones production during *in vitro* growth of Turkish isolates of *Ascochyta rabiei*. After culture filtrates were passed through the C18 cartridge, the solanapyrones were eluted with 2 ml acetonitrile. Quantitation of solanapyrones was determined with LC/MS analyses. Maximum solanapyrones production of the isolates was observed on 14th day of incubation. Therefore, quantitation of solanapyrones of the rest 63 isolates of *A. rabiei* was also determined on the 14th day. Of the 67 *A. rabiei* isolates used in the present study, it was determined that 66 (98.5 %) isolates produced solanapyrone A, 18 (26.9 %) isolates produced solanapyrone B and 64 (95.5 %) isolates produced solanapyrone C. Toxicity of solanapyrones on both sensitive (ILC 1929) and resistant (ILC 3279) chickpea cultivars were demonstrated by the living cell bioassay. The LD_{50} concentrations for solanapyrone A, B and C in the bioassay for the sensitive cultivars were respectively 18.6, 23.2 and 96.8 µg/ml while those for the resistant cultivars were respectively 34.5, 36.2 and 109.3 µg/ml. The LD_{50} concentrations of the mix of solanapyrones in the sensitive and resistant cultivars were respectively 42.4 and 45.4 µg/ml.

Keywords: *Ascochyta rabiei*, Pathotype, Chickpea, Solanapyrones, Bioassay

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most extensively grown legume crops in Turkey. According to FAO records in 2010, total plantation of chickpea is 446218 hectares, production is 530634 tones and rate of yield is 1189 kg hectare^{-1} in Turkey (FAOSTAT, 2012). Chickpea production and quality are negatively affected by a number of biotic and abiotic stresses (Singh et al., 1994). One of the greatest biotic stresses reducing potential yield in chickpea is Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. (Singh and Reddy, 1996). *A. rabiei* is a heterotallic *Ascomycete* with two mating types, and both mating types are present in most chickpea production areas (Trapero-Casas and Kaiser, 1992; Kaiser and Kusmenoğlu, 1997). Pathogenic variability of *A. rabiei* is enhanced by the presence of the teleomorphic stage (Kaiser, 1997), which has been reported from almost all chickpea producing countries in the world (Udupa and Weigand, 1997; Jamil et al., 2000; Maden et al., 2004; Chen et al., 2004; Chongo et al., 2004; Türkkan and Dolar, 2009). *A. rabiei* attacks all above-ground parts of plants and causes necrotic lesions which are circular on leaves and pods and elongate on petioles and stems. When stems and petioles are girdled, they usually break (Nene and Reddy, 1987). The disease may cause total yield loss if the enviromental conditions are favorable (Singh and Reddy, 1990).

Phytotoxins are known to be secreted by a number of phytopathogenic fungi (Durbin, 1981). It is thought that these toxins are playing an important role in plant diseases, because they can induce most or all of the disease symptoms (Yoder, 1980; Strobel, 1982). It is known that early symptoms of *A. rabiei* causes epinasty and loss of turgor in petioles and young branches (Alam et al. 1989). Later, the whole aerial part of the plant may dry out and die. Solanapyrone toxins (A and C) in culture filtrates of *A. rabiei* firstly determined by Alam et al. (1989). After

Chen et al. (1991) optimized solvent system for seperation of solanapyrones, solanapyrone B was also found in culture filtrate of the fungus. In further studies, the workers reported that production of solanapyrone toxins varied according to content of the liquid culture medium on which the fungus was grown (Chen and Strange, 1991; Höhl et al., 1991; Latif et al., 1993; Kaur, 1995; Bahti and Strange, 2004). Application of solanapyrone toxins were reported to led to morphological changes in chickpea leaves and caused brekage in chickpea stem (Höhl et al., 1991; Hamid and Strange, 2000). Moreover, Höhl et al. (1991) observed that 100 and 200 µM concentrations of solanapyrone toxins caused a pronounced bleaching of the chlorophyll in the area of droplet application on leaves. Therefore, it was suggested that such symptoms could result from solanapyrones which were synthesized by *A. rabiei*. Zerroug et al. (2007) determined that 250 µM (75.5 µg ml^{-1}) concentration of solanapyrone A inhibited the root growth of chickpea seedlings by 50%. Hamid and Strange (2000) reported that bleaching of the stem occurred and shoots of chickpea broke just below the uppermost leaf after application of 45.3 µg solanapyrone A. Moreover, they determined that LD_{50} values of solanapyrone toxins varied widely depending on cultivar and solanapyrone A was the most toxic of the three solanapyrones.

The present study was carried out to determine the presence of the solanapyrone toxins in Turkish isolates of *A. rabiei* and toxicity of solanapyrones on both sensitive (ILC 1929) and resistant (ILC 3279) chickpea cultivars.

MATERIALS AND METHODS

Growth, spore production and storage of *Ascochyta rabiei*

Totally sixty-seven isolates of *A. rabiei*, sixty-four of which were obtained from the culture collection of the Department of Plant Protection, Faculty of Agriculture, University of Ankara, were used in this study. The rest of the isolates representing the pathotypes of *A. rabiei* were provided by Dr. Bassam Bayaa from ICARDA. The isolates maintained on microbank were grown on CSMDA (Chikpea Seed Meal Dextrose Agar: chickpea meal 40 g, dextrose 20 g, agar 20 g, distilled water 1L) for 14 days at 22 ± 1 ^{o}C with a 12 h light-photoperiod. The cultures were flooded with sterile distilled water (SDW) and spores were scraped with sterile glass spatula. After the spores were filtered through filter paper to remove mycelial fragments, they were centrifuged at 10000xg/rpm for 10 min. They were resuspended in SDW and centrifuged twice more before finally resuspending them at 1×10^7 spores/ml. They were stored as a final suspension in 10% glycerol at -80 ^{o}C for use in the future experiments.

Toxin Production

A. rabiei isolates were grown on Czapek Dox medium supplemented with metal cations ($ZnSO_4.7H_2O$, 0.05 g l^{-1}, $CaCI_2.2H_2O$, 0.1 g l^{-1}, $CuCI_2.2H_2O$, 0.02 g l^{-1}, $CoCI_2.6H_2O$, 0.02 g l^{-1}, $MnCI_2$.4H2O, 0.02 g l^{-1}) (Hamid and Strange, 2000). The medium was dispensed in 250 ml Erlenmeyer flasks containing 30 ml Czapek Dox medium. Each flask was inoculated with 30 µl spor suspension of *A. rabiei* (10^7 spores ml^{-1}). Flasks were incubated at 20 ± 1 ^{o}C without shaking. Fungal mycelia and spores were removed by filtration through Whatman No. 1 filtrate paper, 7, 14 and 21 days after incubation. Mycelial mass was dried at 70 ^{o}C until constant weight (UNB 500 Oven – 108 lt, “Memmert GmbH + Co. KG”, Germany). Solid phase extaction cartridges (SPE; 1 g C18, end-capped Isolute, Alltech Chromatography, USA) were conditioned with methanol (MeOH) (HPLC grade) (5 ml) and then distilled water (5 ml). Culture filtrates (10 ml) were passed through the column, and after washing with distilled water (5 ml), the toxins were eluted with 2 ml acetonitrile (ACN) (HPLC grade) and stored at -20 ^{o}C until required (Bahti and Strange, 2004).

LC/MS Analysis

The LC analyses for the screening and quantitation of solanapyrone A, B, and C were performed by an Agilent 1100 HPLC system (Waldbronn, Germany) with Agilent 1100 MS detector equipped with ESI interface. The analytical separation was performed on a ACE 5 C18 (150 x 4.6 mm, 5 µm) using the isocratic mixture of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid [A] and acetonitril [B], [A:B] (50:50; v:v) at a flow rate of 0.8 ml min^{-1}. MS data acquisition was obtained with positive and negative-ion detection in selected ion monitoring (SIM) mode.

Growth of chickpeas for bioassay study

Chickpea plants consisting of ILC 1929 (susceptible) and ILC 3279 (resistant) were used in bioassay studies. The seeds were surface sterilized with sodium hypochloride (1%) for 3 min, then washed with sterile distilled water (SDW) for 3 times and, they were sown in 14 cm pots containing sterilized mixtures of soil, sand and fertilizer (1:1:0.5, v/v/v). Plants were grown at 22 ± 1 °C with a 14 h light-photoperiod (light intensity, 260 µmoles sec^{-1} m^{-2}) for 14 day.

Bioassay

Solanapyrone A, B, C and the mix of solanapyrones (1:0.1:1) were predissolved in MeOH and then diluted in Czapek Dox containing 5% dimethyl sulfoxide (DMSO) to yield concentration of 250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 µM.

Living cell bioassay

Living cell bioassay was performed using the method developed by Shohet and Strange (1989). Chickpea plants were watered 30 min prior to cell isolation. Leaflets excised from chickpea plants were cut into small pieces and vacum infiltreted with an enzyme cocktail solution. The enzyme cocktail solution consisted of Cellulase R10, 200 mg (Sigma Aldrich Co., USA); Macerozyme R10, 30 mg (Sigma Aldrich Chemie, Germany) and bovine serum albumin, 5 mg (BSA, Sigma Aldrich Chemie, Germany) in 10 ml of a holding buffer consisting of citric acid monohydrate, 10.5 g l^{-1}; $CaCl_2.2H_2O$, 5 mM; K_2HPO_4, 1 mM; $Mg(SO4)_2.7H2O$, 1 mM; glucose, 100 g l^{-1}; NaOH, 6.2 g l^{-1}, adjusted to pH 5.8 with HCl 0.1 M). The suspension of leaflets in the digestion solution was stirred on a magnetic stirrer at 120 rpm for 20 min. The suspension was filtered through four layers of muslin and then washed three times by centrifuging at 750 rpm for 5 min in the holding buffer. Cell viability was checked by vital staining with fluorescein diacetate (FDA). Solutions at different concentrations of solanapyrones were placed in wells of microtest plates (50 µl/well), and 50 µl of cell suspension was transferred into each well. After cells were incubated at 25 °C for 3 h in the dark, the cells were stained with FDA. Then, 30 µl of cell suspension was transferred onto a microscope slide, and the viability of 50 cells was assessed under a fluorescence microscope (cells with intact plasma membranes fluoresced yellowgreen, while dead cells remained unstained). The LD_{50} values extracted from graphs of probit percent cell death corrected for control values. The experiment was carried out with three replicates.

RESULTS AND DISCUSSION

A number of pathogenic fungi produce one or more toxic metobolites which are injurious to plants (Durbin, 1981). It is thought that these toxins are playing an important role in plant diseases, because they can induce most or all of the disease symptoms (Yoder, 1980; Strobel, 1982). *Ascochyta rabiei* which produces solanapyrone A, B and C as well as chytochalasin D in liquid culture medium causes epinasty and loss of turgor in petioles and young branches on chickpea (Alam et al., 1989; Höhl et al., 1991; Latif et al., 1993). Conventionally, solanapyrone toxins have been determined by HPLC which is based on C18 silica column separation with ACN/water (1:1) mobile phase with UV detection (Chen et al., 1991). As has been reported by other researchers, solanapyrone A was also well separated with UV detection in our study (Chen et al., 1991; Kaur, 1995). However, solanapyone B and C was coeluted so that they had the same retention time (Figure 1a). This method was not sufficiently separated solanapyrones for quantitative analysis and need optimization (Chen et al., 1991). Therefore, solanapyrone A and C toxins were firstly reported from liquid culture filtrate of *A. rabiei*, but solanapyrone B was not determined in culture filtrate of the fungus (Alam et al., 1989). After application of solvent optimization to the separation of solanapyrones toxins, all three solanapyrone A, B and C was isolated from *A. rabiei* (Chen et al., 1991). However, solvent optimization is still limited owing to the difficulty of monitoring the crossing over of peaks in different solvents and the large number of variables which affect the separation (Synder and Kirkland, 1979). The determination and quantification of solanapyrones in the culture filtrate of *A. rabiei* was carried out with chromatography (TLC, HPLC, MS and ^{1}H-NMR) (Höhl et al., 1991). In our study, therefore, the amounts of solanapyrone A, B and C in the culture filtrates of sixty-seven *A. rabiei* isolates were determined by HPLC\MS

analyses. After chromatographing toxins by HPLC using a diode array detector (DAD), UV spectral data of the solanapyrone standarts were obtained (Table 1; Fig. 1). The retention time of solanapyrone A, B and C standards were approximately 11.39, 10.06 and 10.11 min, respectively. Detection was carried out with ESI-MS in SIM mode. Molecular ions selected for quantification of toxins in samples were 303 (positive ion), 287 (positive ion) and 331 (negative ion) for solanapyrone A, B and C, respectively (Fig. 1b, c and d).

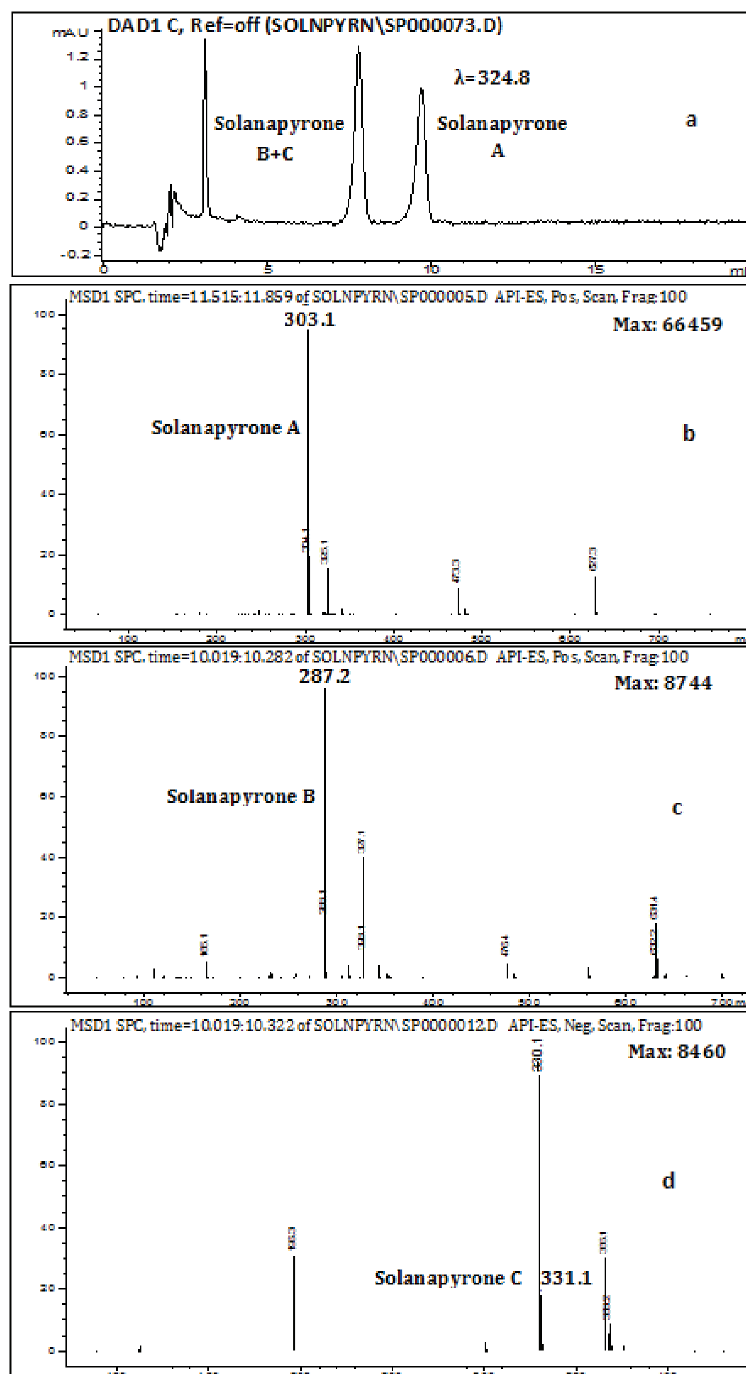

Figure 1. Shows UV and LCMS separation of solanapyrones a) separation of solanapyrone A,B and C by DAD; separation of solanapyrone A (b), B (c) and C (d) by MS

In order to determine kinetics of the solanapyrones production during *in vitro* growth of Turkish isolates of *A. rabiei*, randomly selected four isolates were grown on Czapek Dox liquid medium supplemented with metal cations for 7, 14 and 21 days under continuous light. It is observed that solanapyrones production of the isolates varied considerably during three different incubation periods (Table 2). In the 7th day, production of each three solanapyrones was in very low quantity. The amount of solanapyrone C and B in liquid culture was higher than that of solanapyrone A on this incubation period. Similarly, Höhl et al. (1991) reported that the major toxin in fluids of germinating spores was solanapyrone C, and solanapyrone B was detected in trace amount on the 4th day along with solanapyrone C. In the same period, they did not found solanapyrone A in culture filtrate and it was observed after 6th day of incubation. Results of our study showed that the concentration of solanapyrone A and C in culture filtrates of four isolates (except for solanapyrone C production of isolate Ank-3) reached the highest quantity on the 14th day. This result is in agreement with previous studies reporting that the concentration of solanapyrone A in the culture filtrates peaked at 14-16 day (Bahti and Strange, 2004; Zerroug et al., 2007). In the present study, however, solanapyrone B could not be detected in the culture filtrate of the isolates on the 14th day. In following incubation period, solanapyrone toxins in the fungal culture rapidly decreased. As mentioned by previous researchers, these results show that production of solanapyrone toxins are limited to a certain stage of growth cycle and can imply that the increase of mycelial mass of the fungi is closely related with the quantity of solanapyrones (Höhl et al., 1991; Chen and Strange, 1991; Kaur, 1995). In our main study, therefore, 14th day of incubation was used for extraction of the rest 63 isolates of *A. rabiei*. The amounts of solanapyrone A, B and C of all 67 isolates were shown in Table 3. Although solanapyrone B in culture filtrates of all four isolates in previous study limited to 7th day of the incubation periods, we determined that 18 *A. rabiei* isolates out of 67 produced solanapyrone B on 14th day in the following study. Except for isolate Ank-9, Solanapyrone B quantity of these isolates was also very low compared to the other solanapyrones. Höhl et al. (1991) detected that the concentration of sugar in liquid culture medium especially affected the amount of solanapyrone B whereas solanapyrone A and C remained unaffected. The amount of solanapyrone A and C also varied according to isolates and 64 *A. rabiei* isolates produced both solanapyrone A and C. These toxins weren't detected in culture filtrate of isolate Kay-2. All other isolates produced solanapyrone A and solanapyrone C and they were determined in culture filtrates of 64 *A. rabiei* isolates, except Kay-2, Ams-2 and PII.

Table 1. UV data for solanapyrone A, B and C

Solanapyrone A	λ_{max}: 231, 326 nm
Solanapyrone B	λ_{max}: 200, 302 nm
Solanapyrone C	λ_{max}: 237, 317 nm

Table 2. The amounts of solanapyrone A, B and C in the mycelial dry weight (g) of four *Ascochyta rabiei* isolates

Names of the isolates	**Incubation periods (day)**	**Solanapyrone (µg/g)**		
		A	**B**	**C**
	7	0.09	1.47	1.29
Afy-1	14	15.79	-	9.41
	21	0.13	-	0.82
	7	-	0.28	0.78
Dez-5	14	7.16	-	19.72
	21	0.35	-	9.78
	7	0.08	0.4	1.12
Ank-3	14	38.8	-	0.05
	21	0.90	-	4.27
	7	0.12	0.93	1.50
Kmar-1	14	6.22	-	14.96
	21	1.73	-	1.79

Table 3. The solanapyrone production of sixty seven isolates of *Ascochyta rabiei*

Name of the isolates	**Solanapyrone (µg/g the mycelial dry weight)**				**Pathotypes****
	A	**B**	**C**	**Total**	
***Pathotype I**	22.2	-	4.23	26.43	I
Ady-1	14.43	-	7.39	21.82	I
Ady-2	45.88	-	70.0	115.88	I
Ady-3	1.03	-	9.50	10.53	I
Ady-4	28.92	1.08	80.00	110.00	I
Ady-5	86.67	-	102.5	189.17	I
Ady-6	97.84	-	107.3	205.14	I
Afy-1	15.79	-	9.41	25.20	I
Ams-1	3.97	-	18.26	22.23	I
Ams-2	0.13	-	-	0.13	I
Ank-1	0.84	-	0.95	1.79	I
Ank-2	90.94	-	124.06	215.00	I
Ank-3	38.80	-	0.05	38.85	I
Ank-4	11.8	-	40.82	52.62	I
Ank-5	47.44	-	101.86	149.30	I
Ant-1	5.66	0.08	0.23	5.97	I
Ant-2	7.35	-	4.51	11.86	I
Ant-3	57.20	-	39.56	96.76	I
Çor-1	16.29	0.14	22.77	39.20	I
Çor-2	24.40	0.26	21.76	46.42	I
Çor-3	83.13	2.50	186.56	272.19	I
Dez-1	0.510	-	10.02	10.53	I
Dez-2	49.10	0.33	83.08	132.51	I
Diy-1	20.74	-	59.26	80.00	I
Diy-2	5.16	-	58.39	63.55	I
Diy-3	56.47	-	121.18	177.65	I
Esk-1	0.75	-	33.40	34.15	I
Esk-2	93.55	-	91.29	184.84	I
Esk-3	15.81	-	106.13	121.94	I
Esk-4	16.36	1.09	39.45	56.90	I
Kmar-1	6.22	-	14.96	21.18	I
Kay-1	10.17	-	9.87	20.04	I
Kır-1	18.30	5.11	28.3	51.71	I
Kır-2	0.88	-	5.33	6.21	I
Tok-1	46.50	-	63.00	109.5	I
Uşk-1	2.28	0.15	21.02	23.45	I
Uşk-2	11.47	-	13.53	25.00	I
Uşk-3	16.74	-	14.91	31.65	I
Yoz-1	11.61	-	3.94	15.55	I
***Pathotype II**	9.84	0.05	-	9.89	II
Dez-3	12.61	-	174.78	187.39	II
Kay-2	-	0.08	-	0.08	II
Urf-1	38.52	-	61.85	100.37	II

***Pathotype III**	27.05	-	12.28	39.33	III
Ady-7	9.38	0.11	5.71	15.20	III
Ady-8	16.82	-	3.41	20.23	III
Ams-3	0.05	0.10	0.28	0.43	III
Ams-4	13.34	-	44.84	58.18	III
Ank-7	43.20	0.16	34.33	77.69	III
Ank-6	23.93	-	3.03	26.96	III
Ank-8	20.57	-	20.53	41.1	III
Ank-9	13.85	41.54	133.08	188.47	III
Bur-1	26.15	-	361.54	387.69	III
Çor-4	43.19	-	69.86	113.05	III
Dez-4	0.22	-	15.43	15.65	III
Dez-5	7.16	-	19.72	26.88	III
Dez-6	24.36	-	18.05	42.41	III
Dez-7	9.80	-	100.40	110.20	III
Diy-4	52.41	-	66.55	118.96	III
Diy-5	10.52	0.11	4.16	14.79	III
Diy-6	2.72	-	13.97	16.69	III
Diy-7	5.46	0.08	12.20	17.74	III
Siv-1	3.92	-	22.94	26.86	III
Uşk-4	14.55	-	47.27	61.82	III
Uşk-5	4.10	-	22.37	26.47	III
Yoz-2	34.68	0.07	80.96	115.71	III
Yoz-3	5.44	-	26.83	32.27	III

* Referans pathotypes of *A. rabiei* were provided by Dr. Bassam Baya.
** Türkkan and Dolar (2009)

These results are in agreement with studies of Chen and Strange (1991), who reported that each three solanapyrones were produced by *A. rabiei* when it is grown on Czapek Dox medium supplemented with metal cations.

Pathogenic variability among isolates of *A. rabiei* has been reported from almost all chickpea producing countries in the world including India, USA, Syria, Pakistan, Turkey and Canada. The isolates used in these studies have been classified into 3 to 17 pathotypes based on their reactions on 3 to 16 host genotypes (Udupa and Weigand, 1997; Jamil et al., 2000; Maden et al., 2004; Chen et al., 2004; Chongo et al., 2004). Recently, Türkkan and Dolar (2009) categorized 64 *A. rabiei* isolates into three pathotypes based on differences in aggressiveness on three differential chickpea cultivars (ILC 1929, ILC 482 and ILC 3279). Pathotype I was the least agressive pathotype, whereas pathotype III was the most agressive pathotype. Because the same isolates were used in present study, we could compare solanapyrone production among all three pathotypes. Although solanapyrones production of some of pathotype I isolates was very low in liquid culture medium, the others' was very high. Moreover, higher amounts of solanapyrone toxins were not isolated from the more aggressive isolates of pathotype II and III. For example, solanapyrones production of isolates Kay-2 and Ams-3 which are belonging to PII and PIII, respectively was very low. Results of our study therefore showed that there were not a correlation between pathotypes of *A. rabiei* isolates and their solanapyrone production *in vitro* conditions. Similar results were observed by Latif et al. (1998), who determined that the isolates produced significant levels of the solanapyrones *in vitro* growth and also induced significant levels of phytotoxic compounds in fungus infected plants caused a moderate degree of disease severity. Therefore, they reported that production of phytotoxic compounds had no bearing on the disease severity or fungal virulence because of the discrepancy in the levels of production of toxins during *in vitro* and *in vivo* situations. Sugawara and Strobel (1987) reported that a phytotoxin involved in a host–parasite interaction should be demonstrable in susceptible host plants after infection. Although Shahid and Riazuddin (1998) suggested that solanapyrone C has been found in field infected plants, other researchers could not detect any of solanapyrone toxins in chickpea tissue infected by the fungus (Höhl et al., 1991; Hamid and Strange, 2000; Bahti and Strange, 2004). However, they reported that application of the different toxin concentrations to chickpea plants led to morphological changes in both leaf and stem structures that are well comparable to those observed in the tissue of chickpea plants when invaded by the fungus. Therefore, it is argued that solanapyrones isolated from

A. rabiei may contribute to virulence or may be necessary for pathogenicity owing to their affects on chickpea plants (Alam et al., 1989; Höhl et al., 1991; Latif et al., 1993; Hamid and Strange, 2000).

In our study, the effects of solanapyrone toxins on both susceptible and resistant chickpeas were determined with a living cell bioassay. It was found that solanapyrone A (18.6 and 34.5 µg ml^{-1} LD_{50} values for susceptible and resistant cultivars, respectively) was more toxic than solanapyrone B (23.2 and 36.2 µg ml^{-1}) and C (96.8 and 109.3 µg ml^{-1}) on each two chickpea cells as well as the mix of solanapyrone A, B and C (42.4 and 45.4 µg ml^{-1}). This result is in agreement with previous studies, reporting that LD_{50} values of solanapyrone toxins varied widely depending on cultivar and solanapyrone A was the most toxic of all the three solanapyrones (Hamid and Strange, 2000). Morover, the researchers reported that the effect of solanapyrone A, B and C on cells isolated from chickpea was additive rather than synergistic (Alam et al., 1989; Höhl et al., 1991; Latif et al., 1993).

ÖZET

ASCOCHYTA RABİEİ'NİN TÜRK İZOLATLARININ SOLANAPYRONE ÜRETİMİ VE NOHUTLAR ÜZERİNDEKİ FİTOTOKSİTELERİ

Ascochyta rabiei izolatlarının solanapyrone A, B ve C üretim kinetiğini belirlemek amacıyla rastgele seçilmiş 4 izolat 7, 14 ve 21 gün süreyle inorganik tuzlarla zenginleştirilmiş Czapek Dox sıvı besin ortamında geliştirilmiştir. Kültür fitratları C18 isolute kartijlerinden geçirildikten sonra solanapyrone toksinleri 2 ml acetonitril ile elute edilmiştir. Toksinlerinin kantitatif miktarları LC/MS ile belirlenmiştir. İzolatların solanapyrone A, B ve C üretiminin maksimum olduğu inkübasyon periyodunun 14. gün olduğu belirlenmiştir. Bu nedenle geri kalan 63 izolatın solanapyrone A, B ve C miktarları inkübasyonun 14. günü esas alınarak belirlenmiştir. Çalışmada kullanılan izolatların 66 (% 98.5)' sının solanapyrone A, 18 (% 26.9)' inin solanapyrone B ve 64 (% 95.5)' ünün solanapyrone C ürettiği belirlenmiştir. Solanapyrone toksinlerinin toksisitesi hassas ve dayanıklı bitkilerde canlı hücre bioassay çalışmaları ile belirlenmiştir. Solanapyrone A, B ve C' nin hassas çeşit ILC 1929' da LD_{50} değerleri sırasıyla 18.6, 23.2 ve 96.8 µg ml^{-1} iken dayanıklı çeşit ILC 3279' da sırasıyla 34.5, 36.2 ve 109.3 µg ml^{-1}' dir. Hassas ve dayanıklı çeşitlerde solanapyrone toksinlerinin karışımının LD_{50} konsantrasyonları sırasıyla 42.4 ve 45.4 µg ml^{-1}'dir.

Anahtar Sözcükler: *Ascochyta rabiei*, Pathotype, Chickpea, Solanapyrones, Bioassay

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