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Elicitation of Phytoalexins against Late Blight Disease in Tomato Plants by Abiotic Agents and its Impact on Disease Resistance

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Tomato is one of the world's major vegetable crops. Like other important crops, tomatoes also suffer huge crop losses due to many diseases. Among these diseases, late blight caused by *Phytophthora infestans*. The present study was conducted to induce defence reaction in tomato plants against late blight disease under field conditions using some abiotic agents. Tomato plants sprayed by ethyl salicylic acid (2.5 ml/20 litre water) resulted in lowering disease incidence to great extent (from 25±1.41% in control plants to 1.66±1.03%), followed by coconut milk solution (1%) and Agrispon® (1%). These three agents were found to induce accumulation of sesquiterpenoid stress metabolites (phytoalexins) in tomato fruits when they were tested for its elicitation capability. Rishitin, lubimin, phytoberin and unknown compound were detected in tomato fruit diffusates treated by abiotic agents. This is the first record that these agents elicit phytoalexins in tomato fruits.

Keywords: Induced resistance, late blight, phytoalexins, sesquiterpenoid and tomato.

Phytoalexins (PA) are low molecular weight compounds that play a considerable role in plant disease resistance (Elgersma and Liem 1989; Hammerschmids, 1999; Tsuneo, 2005 and Mostafa and Gado, 2007). Synthesis of phytoalexins (PA) in plants could be induced by different biotic and abiotic factors. (Bostock *et al.*, 1981; Baily, 1982; Kroon *et al.*, 1991; Sundaresan *et al.*, 1993; Nojiri *et al.*, 1996; Kuchitsu *et al.*, 1993 and Umemura *et al.,* 2002).

It was established that tomato plants accumulate sesquiterpenoid phytoalexins as the result of infection by *Phytophthora infestans* in case of plant resistant (Stoessl *et al.*, 1978). Moreover, Elgersma *et al.* (1984) have isolated trimethysilyl derivative in response to infection of tomato plants by *Verticillium albo-atrum*.

Late blight on tomato plants caused by the Oomycetes *Phytophthora infestans* considered is as one of the most important diseases of tomato in open fields and under greenhouse conditions (Fry and Goodwin, 1995; Suijkowski *et al.*, 1996; Fry and Goodwin, 1997 and Yongjun, *et al.*, 2010). This serious disease actually disperses very quickly, and therefore it's difficult to be controlled.

Induction of systemic acquired resistance in plants became widespread nowadays to manage broad range of plant pathogens including bacteria, viruses, fungi and some insects (Sticher *et al.*, 1997) by application of many chemical compounds, *i.e*. salicylic acid or its derivatives (Dean and Kuc, 1987 and Molinari and Loffredo, 2006). In addition, plant extracts were found to induce resistance in plants (Daay *et al.*, 1995; Gado, 2007; Mostafa and Gado, 2007 and Mostafa *et al.*, 2007).

In the previous study of Mostafa and Gado (2007), different abiotic agents were found to induce of PA in potato plants and in enhanced plant disease resistance against late bight pathogen. Therefore, the previously tested agents on potato plant were retested on tomato plants against late blight disease caused by *Phytophthora infestans* in relation to elicitation of PA in tomato fruits.

Materials and Methods

Study site:

A field (1/2 feddan) located in El-Dair village, Shebin El Kanater District, Qualubeya Governorate cultivated by tomato plants (cv. Prichard) was chosen to carry out this experiment during growing season (February-June, 2009). This location was chosen because it has long history of late blight epidemics on both potato and tomato plants. Field was divided into 8 plots, every plot contained at least 7 rows (replicates), and every row contained at least 20 plants. Every plot was sprayed by a particular water solution of abiotic agents when plant reached 60 days old.

Tested agents:

The following 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).

Every plot was sprayed with 20 litres water solution of abiotic agents when plants reached 60 days old, and then repeated after ten days.

Disease assessment:

Visual observation of late blight symptoms Fig. (1) reveal that disease severity was approximately similar on all diseased plants; therefore, the average percentage of disease severity was recorded at the end of study according to (James, 1971). Standard deviation was calculated, (Ghahramani, 2000). It could be mentioned that results of border rows were neglected.

Testing of abiotic factors as elicitors of phytoalexins in tomato fruits:

Tomato fruits (cv. Prichard) at the beginning of maturation were harvested, washed several times by tap water, surface sterilized in 1% potassium hypochlorite solution for 5 min. then washed in sterilized distilled water. Each fruit was cut transversely, and seeds were removed from fruit cavities (Fig. 2), then cavities were washed by sterilized water and left for dryness.

Halves of tomato fruits were preserved in a moist chamber and the cavities were filled with particular tested agents then incubated at 22±1ºC. Solutions were left for 48 h then harvested (Fig.2). Diffusions were centrifuged at 10000 rpm for 10 min. then mixed with chloroform (1:5 v/v). Chloroform phase was separated, evaporated under reduced pressure then the residues were redissolved in 1 ml chloroform. A spots (200µl) were spotted on silica gel-G plates (20x 20 cm) and subjected for chromatographic separation. A system consisted of chloroform : methanol (85:15 v/v) was used for PA separation. Selica Gel plates were dried, and then sprayed with a specific reagent of sesquiterpenoid compound [acidified vaniline in methanol (Stoessl, 1982)]. Plates were heated at 55ºC for 5 min. to visualize PA (Metlitski *et al.,* 1976).

Results

When environmental condition became favourable for late bight incidence, typical symptoms of late bight started to appear on some plants (Fig. 1), this was observed when plants reached 65 days old.

Assessment of disease severity of blight revealed that no differences in disease severity was observed between plants, therefore after 65 days, disease severity of late blight was determined by the end of season. Data presented in Fig. (4) show that the average percentage of diseased plants in untreated blots reached 25±1.41%. Ethyl salicylic acid solutions gave the best results in controlling such disease, disease incidence reached 1.66±1.03% coconut milk solutions come in the second order, it gave 6.65±2.2% diseased plants. Agricultural stimulants solutions, *i.e.* Sincocin and Agrispon gave 10.72±2.24% and 6.18±2.12%, respectively. Results indicated that the efficiency of ethyl salicylic acid in controlling late blight disease on tomato plants reached 93.36% followed by coconut milk solution and the least efficiency was obtained by mango malformed inflorescence extract.

Phytoalexin elicitation by tested abiotic agent:

Separation of tomato fruit phytoalexins using TLC (Fig. 3) gave four compounds, *i.e.* Rishitin (Rf 0.21), Lubimin (Rf 0.41), phytubrin (Rf. 0.67) and unknown compound, gave a positive reaction with acidified vanelic acid with Rf 0.76. The most effective agents for PA production were ESA, COM and SIN in descending order. Other tested agents, *i.e.* JAS, MAN and AGR, elicited PA in very low density.

Fig. 1. Visual observation of late blight symptoms.

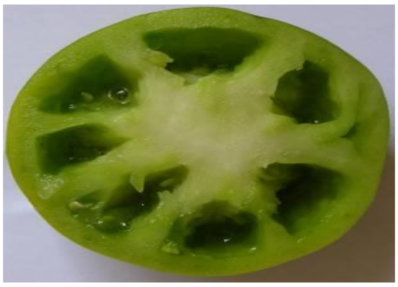

Fig. 2. Elicitors of phytoalexins on tomato fruits halves.

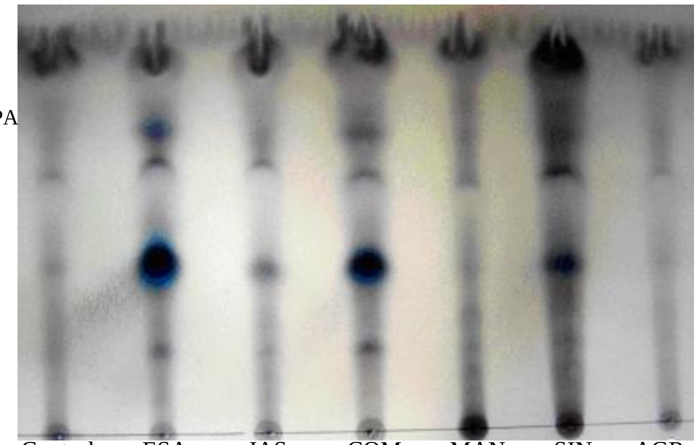

Fig. 3. Phytoalexins elicited by tested agents.

Whereas: 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).

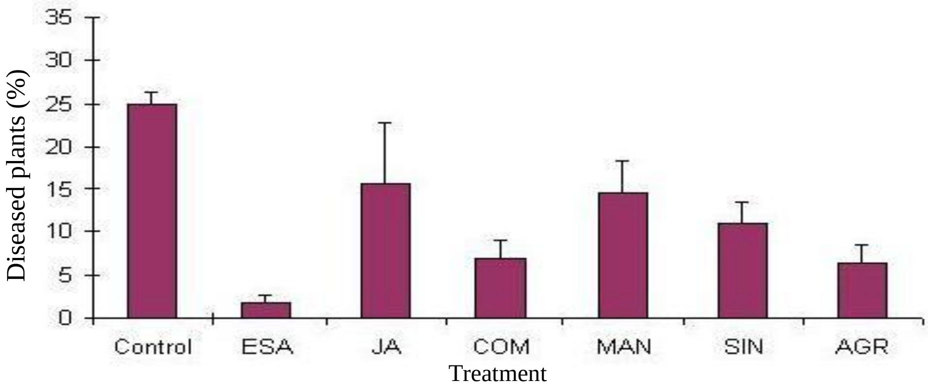

Fig. 4. Effect of chemical agents and natural extracts on percentage of late blight disease.

Whereas: 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).

D i s c u s s i o n

Late blight of tomatoes caused by *Phytophthora infestans* is considered as the most destructive diseases in temperate and semi-temperate region (Suijkowski *et al.,* 1996; Fry and Goodwin, 1997 and Yongjun *et al.,* 2010).

The epidemiology of such disease reveals that under suitable conditions, it spread very quickly and left plant completely collapsed within few days (Fry and Goodwin, 1995).

The only effective method for management of such disease is fungicides, which should be used in suitable time before disease occurring. Otherwise, it is very difficult to control when it start to spread over plants.

The present investigation was claimed to activate plant defence reactions in tomato plants by different abiotic agents. Therefore, a field of tomato in area has long history of late blight epidemic was chosen in order to carry out this study.

It could divide the abiotic tested agents in two categories: chemical compounds (*i.e.* ethyl salicylic acid and Jasmonic acid), and plant extracts (i.e. coconut milk solution, water extract of malformed mango inflorescence, Agrispon® and Sincocin®). These agents were sprayed on tomato plants before the appearance of the first sign of disease symptoms.

Ethyl salicylic acid solution (0.125 ml/litre) was the best agent, it reduced disease incidence from 25±1.41% (non treated plants) to 1.64±1.03%. Coconut milk solutions and Sincocin came in the next descending order; it gave 6.18±2.12% and 6.65±2.2%, respectively. Mango malformed inflorescence extract over lied in the late order.

Inducing resistance in plants is referred to activation of different factors (*i.e.* phytoalexins induction, PR proteins, enhancement of peroxidase activity, deposition of lignin … etc.), (Kuc, 1972; Keen and Little field 1979; Kuc, 1982a; Kuc, 1982b; Jeandet *et al.*, 1995 and Ning *et al.*, 2003).

In the present study we have focused on one factor of resistance, *i.e*. the ability of tested agents to elicit sesquiterpenoid stress compounds (phytoalexins) in plants and their impact on disease resistance. In this regard, many authors speculated and proved that PA plays a considerable role in plant disease resistance (Kuc, 1972; Baily, 1982; Thomzik *et al.*, 1997; Mert-turk, 2002; Soylu *et al.*, 2002; Umemura *et al.*, 2002 and Mostafa and Gado, 2007).

In many studies, cell suspension culture was used as a model for studying the phytoalexins elicitation activity of different elicitors (Glazebrook and Ousubel, 1994; Nojiri *et al.*, 1996 and Chen and Chen, 1999).

In the present study, a new technique was adopted. Tomato fruit at the early stage of maturation were evacuated from seeds, and seeds chambers were used as cells for studying elicitation activity of tested abiotic agents of PA. Sesquiterpenoid phytoalexins were extracted from diffusates of seed cells previously filled with tested agent and subjected to TLC analysis. A specific reagent for solanacious PA was used for visualization of PA (Stoessl *et al.*, 1978 and Stoessl, 1982). Ethyl salicylic acid, coconut milk and Sincocin® were found to have elicitation activity. They elicited rishitin, lubimin, phytuberin and unknown sesquiterpenoid compound gave a positive reaction with vaniline sulphuric acid reagent. In this respect, Stoessl *et al.* (1978) have predicted that tomato may produce PA other than rishitin. In 1984, Elgersma *et al.* discovered new phytoalexins falcarinal and falcarindiel in tomato plants after they had been infected by *Verticillium albo-atrum* and identified trimethysilyl derivatives of four PA using capillary chromatography mass spectrometry.

In our study, ethyl salicylic acid, coconut milk and Sincocin® solution were found to be highly active as PA elicitors comparing with other agents and in the same time, they gave the best results in controlling tomato late blight under field conditions, Ethyl salicylic acid had a high PA elicitation activity and it gave the best result in disease control.

These result are in accordance with the result obtained in our previous study (Mostafa and Gado, 2007) whereas, the tested abiotic agents elicit PA in potato plants and enhanced resistance against late bight disease.

Other tested agents, *i.e.* Jasmonic acid, mango malformed inflorescence extract and Agrispon® induces moderately degree of resistance in tomato against late blight and they hadn't ability to act through PA induction. Therefore, their effect as inducing agent of disease resistance may be went via other routs than PA induction, and this need further study.

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تحفيز أنتاج الفيتوألكسينات ضد مرض الندوه المتأخرة فى نباتات الطماطم بواسطة عوامل غير حيوية ومردودها على مقاومة المرض

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** قسم البيولوجى – كلية العلوم – جامعة الطائف – السعودية.

الطماطم أحد أهم محاصيل الخضر فى العالم. مثلها مثل محاصيل مهمة أخرى تعانى الطماطم من خسائر كبيرة فى المحصول بسبب الاصابه بالعديد من الامراض. ومن بين هذه الامراض مرض اللفحة المتأخرة المتسبب عن *Phytophthora infestans*.

وقد أجريت هذه الدراسه بغرض حث نباتات الطماطم لمقاومة مرض اللفحة المتأخرة تحت ظروف الحقل بإستخدام مواد لاحيوية.

أدى رش نباتات الطملطم بحامض السالسيليك الإيثيلى (٢,٥ مل /٢٠ لتر) لخفض معدلات المرض إلى حد كبير حيث انخفضت النسبة المؤية للنباتات المصابة بالمرض من ٢٥±١,٤١% فى حالة النباتات الغير معاملة الى ١,٦٦±١,٠٣% فى حالة المعاملة بحمض الساليسيليك الأثيلى الى٦,٦٥±١,٠٣% فى حالة المعاملة بلبن جوز الهند (١%) ، ومحلول Agrispon (١مل / لتر).

ثبت أن هذه المواد الثلاثه تدفع ثمار الطماطم لإنتاج الفيتوالكسينات السسكتربينية: ريشيتين و لوبينين و فيتورين و مركب اخر غير معروف. ويعد ذلك اول تسجيل على ان العوامل اللاحيويه تحفز انتاج الفيتوألكسينات فى ثمار الطماطم.