الاختلافات المرضية و الفسيولوجية و الجزيئية بين عزلات الفطر الترناريا سولانى مسبب مرض اللفحة المبكرة علــى الطماطم

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PATHOLOGICAL, PHYSIOLOGICAL, AND MOLECULAR VARIATIONS AMONG ISOLATES OF *Alternaria solani* THE CAUSAL OF TOMATO EARLY

BLIGHT DISEASE

BY

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B.Sc. (Agirc.), Assiut University (1988)
M.Sc. (Plant Pathol.) , Cairo University (2000)

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Cairo University

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Approval Sheet

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CNTENTES

INTRODUCTION

In Egypt tomato (*Lycopersicon esculentum* Mill.), ranked as the number one vegetable cash crop with total planting area 459,283 feddan which yielded about 7,140,198 ton of fruits (Department of Agricultural Economic Statistical, Ministry of Agriculture and Land Reclamation, March 2005). This area is about 31.5% of the total vegetable cultivated area in Egypt. Globally, Egypt ranked in the fifth position in growing tomato crop. Egyptian consumption per capita is almost equal to the American one. Egyptian climate is favourable for tomato production as well as incidence of early blight disease most of the year.

Tomato is considered as one of the highest nutritional crops because of its high contents of vitamin C as well as many chemical compounds and elements which are not found in the other Solanaceae crops. Tomato plants are vulnerable to attack by many destructive pathogens. Several fungal diseases are affecting tomato plants in all growing stages causing a considerable reduction in fruit yield per feddan.

Alternaria solani (Ellis and Martin) Jones and Grout, is known to be the causal agent of two distinct phases on tomato plants, *i.e*. early blight and collar rot (Pritchard and Porte, 1921). Early blight disease is responsible of most serious problem on tomato than collar rot. Collar rot is mainly a seedbed disease that carried to the field on tomato transplants and has been associated with the southern production of tomato seedlings in open fields. Meanwhile, early blight disease occurs wherever tomatoes are grown, under humid or semiarid climates, with dew or water irrigation, which providing good conditions for disease development (Tsror and Bieche, 1999). The causal agent is an airborne fungus, whose dark multicultural conidia are dispersed by wind and water splash (Jones,

1991). It is well known that sporulation of *A. solani* is very scarce, either on synthetic or on semi-synthetic media, using the conventional methods Benliglu and Delen (1996). The pathogen penetrates wounded leaves, causing typical symptoms of concentric rings that severely damage the foliage, resulting in considerable reduce yield (number of fruits and size)

Because of increasing the importance of early blight disease all over the world, studies for its control are continually applied in different countries such as Brazil (Romeiro *et al*., 2000 and Tofoli *et al*., 2003), Canada (Lynch *et al*., 1991 and Jong *et al.,* 2001), Cuba (Sueiro-Pelegrin *et al.,* 2003), Egypt (El-Abyad *et al.*, 1996; Ismail, 1999 and Ghoma, 2000), Greece (Vloutoglou *et al.,* 2000), Israel (Tsror and Bieche, 1999), Nigeria (Gwary and Nahunnaro, 1998), Romania (Tomescu *et al.,* 2002), SriLanka (Wickramaarachchi *et al.,* 2003), Turkey (Ozcan and Boyraz, 2000) and USA (Gardner and Shoemaker, 1999 and Tietjen *et al.,* 2001).

The present investigation was conducted to address some important issues concerning the nature of the causal pathogen of tomato early blight and/or collar rot diseases as well as different approaches for its control. The first objective was to explore the host/pathogen interaction especially pathogenicity of different *A. solani* isolates and susceptibility of different commercial tomato cultivars to both early blight and collar rot infection. Also, the influence of plant age and ripping stage on the development of tomato early blight disease was investigated. The second objective was to determine the combined effect of different semi-synthetic media and different incubation conditions on the induction of conidial spores of *A. solani.* Also, the capability of the tested isolates to produce *in vitro* mycotoxin substances was studied. Moreover, molecular characteristics,

using random amplified polymorphic DNA (RAPD) analysis, polymerase chain reaction (PCR) technique was followed to define genetic variations Among the tested *A. solani* isolates. Third objective of this investigation was to explore different *in vitro* and *in vivo* approaches for early blight disease control, *i.e*. chemical and biological control. In this concern, different fungicides and/or simple chemical substrates, such as phosphate salts and potassium nitrate, as well as salicylic acid and gibberellic acid, were evaluated for their inhibitory effect against the causal fungus and disease intensity. Also, the capability of different antagonistic fungi and bacteria were evaluated for their effects as biocontrol agents against the causal fungus of tomato early blight disease.

REVIEW OF LITERATURE

1. Globally distribution of tomato early blight disease:

Early blight disease, caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, occurs wherever tomatoes are grown in humid or semiarid climates, with dew or irrigation water providing conditions for disease development (Jones, 1991). The disease was recorded in most of the world countries, *i.e.* Argentina (Monaco *et al*., 1999), Brazil (Romeiro *et al*., 2000; Leite *et al*., 2003 and Tofoli *etal*., 2003), Bulgaria (Stancheva and Stamova, 1990), Cameroon (Fontem *et al.*, 1999 and Fontem, 2003), Canada (Holley *et al.*,1985; Tolman *et al.*, 1986; Srivastava *et al.*, 1989; Fry and Shtieberg, 1990; Lynch *et al*., 1991 and Jong *et al.*, 2001), China (Zhang-GuoQiang *et al*., 2002; Xu-XiangYang *et al*., 2002; Fang-Ling *et al.*, 2002 and Zhang-JianGuo *et al.*, 2003)), Cuba (Castellanos *et al.*, 1989; Andreu-Rodriguez and Cupull-Santana, 1993; Castellanos *et al.*, 1995; Salgado *et al.*, 1999; Gonzalez-Chavez *et al.*, 2003 and Sueiro-Pelegrin *et al.*, 2003), Egypt (Saad and Stino, 1982; Ahmed and Saleh, 1987; El-Abyad *et al*., 1993; Abou-Zeid., 1995; El-Abyad *et al.*, 1996 and Ismail, 1999), Greece (Vakalouakis, 1991 and Vloutoglou *et al.*, 2000), India (Sharma *et al*., 1997; Krishna-Swamy *et al*., 1998; Sawant *et al.*, 1999; Babu *et al*., 2000a; Chhabra *et al.*, 2000; Singh *et al.*, 2000; Suryavanshi *et al.*, 2000; Sawant and Desai, 2001 and Prasad and Naik, 2003), Israel (Shtienberg *et al.*, 1996 and Tsror and Bieche, 1999), Japan (Langsdorf *et al*., 1991; Okamura *et al*., 1996 and Ichihara and Oikawa, 1997), Korea Republic (Lee-HyangBurm *et al.*, 1995) Lithuania (Surviliene *et al.*, 2003) Mexico (Gomez-Rodriguez *et al.*, 2003), Nigeria (Gwary and Nahunnaro, 1998), Romania (Tomescu *et al*., 2002), Republic of Macedonia (Jovancev, 1998), Russia (Kozlovskii

and Kvasnyuk, 1984), SriLanka (Wickramaarachchi *et al*., 2003), Taiwan (Liu-ChienHui *et al.*, 1996 and Liu-ChienHui *et al.*, 1997), Turkey (Benlioglu and Delen, 1996; Delen *et al*., 1996; Ozcelik and Ozcelik, 1997 and Ozcan and Boyraz, 2000), USA (Christ, 1991; Edwards *et al.*, 1996; Louws *et al*., 1996; Goth and Keane, 1997; Krohn *et al.*, 1998; Gardner and Shoemaker, 1999 and Tietjen *et al.*, 2001) and Zambia (Andersson, 1987).

2. The Causal organism:

Many investigators reported that early blight disease, caused by *Alternaria solani*, is considered as the most important foliar disease on tomato (Saad and Stino, 1982; Ahmed and Saleh, 1987; Maiero *et al*., 1990; Vakalounkis, 1991; Abou-Zeid., 1995; Moretto and Barreto, 1995; El-Abyad *et al.*, 1996; Keinath *et al*., 1996; Krishna-Swamy *et al*., 1998; Weir *et al*., 1998; Fontem *et al*., 1999; Ismail, 1999; Tsror and Bieche, 1999 1999; Babu *et al*., 2000a; Foolad *et al*., 2000; Vloutoglou *et al*., 2000; and Christ and Haynes, 2001).

Madden *et al*. (1978) reported that the principal foliar disease of tomato in North Eastern USA is early blight, caused by *Alternaria solani*. The disease characterized by dark lesions with concentric rings, first evident on the lower leaves. Eventually, defoliation becomes pronounced as the diseases progress.

Langsdorf *et al*. (1990) reported that avirulent isolate of *A. solani* was obtained from potato leaves infected by early blight. The fungus was maintained on V-8 juice agar slants and stored at 4^{o}C.