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STUDIES ON SOME POTATO FOLIAR DISEASES

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ABSTRACT: Potato represents one of the most important vegetable crops in family Solanaceae. Several fungal diseases attack the foliage system of potato plants during all growth stages. Late blight caused by *Phytopthora infestans* (Mont.) de Bary and early blight caused by *Alternaria solani* (Ell. and Mart.) Jones and Grout, as well as gray mould incited by *Botrytis cinerea* Person are considered the most destructive potato foliar diseases. Three potato varieties (Dimont, Nicola and Spunta) were found to be susceptible to the three foliage diseases in Sharkyia, Behera or in Qalubyia governorates. Behera was the most favourable governorate for the appearance of all the investigated diseases. Three isolates belong to the three tested pathogens were identified and their pathogenic capabilities were determined. Disease severity and varietal reaction against different pathogens were determined under laboratory and greenhouse conditions using detached and intact leaves technique. This study detected that different tested potato varieties showed different disease severity levels.

Key words: Potato, foliar pathogens, pathogenicity test, varital reaction.

INTRODUCTION

Several fugal diseases can attack the potato foliage during the all stages of the growth. Late blight caused by *Phytophthora infestans* (Mont.) de Bary and early blight caused by *Alternaria solani* Soraur, as well as gray mould incited by *Botrytis cinerea* Person are considered the most destructive diseases for potato plants. Symptoms of these diseases appear first on lower leaves and stems as brown to black spots that gradually enlarge. Close concentric rings within each spot often, but not always identify the early blight disease. Christ and Hays (2001) showed that early blight disease

caused by *Alternaria solani* is one of the most serious diseases of potato attacking foliage system and occurs in most potato growing regions world wide. Gray mould caused by *Botrytis cinerea* is a widespread disease especially under fogy weather conditions. The disease overwinters in plant debris which is the main source of primary inoculum within spring season. Gray mould fungus is found on the older and lower leaves and the fungus grows as gray or brownish growth on all foliage system parts. All leaves are blighted and a soft gray rot may attack the stems (Heneriquez, 2000).

Many plant pathologists all over the world pointed that late blight caused by *Phytophthora infestans* is considered as major disease on potato and tomato (Gavino *et al.*, 2000 and Steven, 2005). *Phytophthora infestans* can attack both tubers and foliage at any stage of growth. When conditions are favorable (Temperature and relative humidity), the fungus can spread rapidly through the crop, causing complete and rapid blight of foliage. The lesions on leaves and stems become visible as small flecks within a few days after infection, expanding to form water soaked, and grey-green area on the leaf. Tubers are infected by spores washed from lesions to the soil and also by contacting spores at harvest. Infected tubers are characterized by patches of irregular, small to large slightly depressed areas of brown to purplish skin. Cutting just below the skin reveals dark, reddish-brown tissues and dry corky rots (Aly *et al.*, 2000). Mayton *et al.* (2001) found that oospores produced in plant were puried in the field in the fall of 1998 were capable to infect both tomato and potato leaflets when recovered in May 1999. Talvoja and Ojarand (2005) stated that assessments made from 2002 to 2004 showed that all tested cultivars (Varajane Kollane (Agriedezeltenie), Maret, Piret, Ando and Anti)retained their characteristics resistance to the phytotypically diverse *P. infestans* population.

Several investigators stated that *Alternaria solani* was the main pathogen of potato early blight (Al-Mughrabi, 2004 and Neelima and Ramunpeet, 2004). Early blight might affect the leaves, stems and fruits or tubers of infected tomato and potato plants and is the most common leaf spot disease in many countries. Characteristic symptoms appear as

a dark, concentric ring of necrotic tissue. Early blight is responsible for a large proportion of total monetary losses sustained by tomato producers every growing season. If disease incidence is high, the fungus can cause extensive defoliation, leading to a reduction in fruit yield (Wiik, 2003). Kaul and Sexena (1988) reported that four cultural groups of *A. solani* causing early blight were distinguished among 92 single spore culture isolates obtained from 34 potato cultivars in Indian States. Vevetia Rodriguez *et al.* (2002) reported that potato cv. Desiree infected with *A. solani* exhibited higher resistance to the pathogen compared to the susceptible cultivar Desiree IBP-10.

Heneriquez (2000) revealed that nowadays, gray mould disease caused by *Botrytis cinerea* is considered as one of the most important fungi which attacks the foliage system of potato. Shtienberg and Elad (1997) stated that *Botrytis cinerea*, the causal pathogen of gray mould can attack leaves, stems, flowers, fruits and tubers of various vegetable crops grown either in the field or in the greenhouse, including tomato and potato but lesions can expand to other tissues (stems). In temperate greenhouses, the occurrence on leaves and fruits of tomato is limited and infections of pruning wounds are more common. O'Neill *et al.* (1997) mentioned that stem infection has become relatively important in recent years. Severe attack detected high incidence of potato plant death especially in long-season crops. Spore concentration was reported to have a critical impact on gray mould incidence and on secondary sporulation after occurrence of infection.

The present work was designed to screen and isolate the causal organisms causing foliage diseases of potato in some Egyptian Delta governorates (Sharkyia, Qalubyia and Behera).

MATERIALS AND METHODS

Disease Survey

Field survey for potato early and late blight as well as gray mould was conducted during two successive growing winter and summer season of 2004 at some Egyptian Delta localities, Sharkyia (El Knaiat village), Qalubyia (Meet El Hofeein village) and Behera (Badr village) governorates.

Three fields in each governorate were inspected and ten random samples of plants were examined to determine the percentage and frequency of each disease.

Isolation and Identification of the Causal Organisms

Samples of potato plants showed disease symptoms (early and late blight as well as gray mould) were collected in icebox from previously mentioned localities and transformed to the laboratory. Collected samples (leaves and stems) were washed thoroughly with tap water, surface sterilized using 5% chlorine solution for one minute, then washed by sterilized distilled water and dried between two sterilized filter papers. Dried sterilized plant materials (leaves and stems) were cut into small pieces using sterilized scalpel and placed on plain agar medium to isolate the causal organisms of early blight and gray mould while selective medium, corn meal agar, (Abd-El-Moity, 1985) was used to isolate the causal organism of late blight. Inoculated plates were incubated at 25- 28 °C. Plates were examined periodically and developed mycelia were picked up and transferred to potato dextrose agar (PDA) medium or corn meal medium according to the causal organism. The isolated fungi were purified using the hyphal tip and/or single spore techniques according to Brown (1924) and Hawker (1960). The purified fungi were transferred to PDA slants or corn meal agar medium. Different slants were incubated at 5°C or 28°C for 7 days according to the causal organism. The developed fungal cultures were stored at 5°C for further studies. The pure cultures obtained isolates were identified at Mycology Department, Plant Pathology Research Institute, ARC.

Isolated fungi of early blight and gray mould were identified according to their cultural and morphological characteristics described by Groves (1946), Gilman (1957), Barnett (1960), Ellis and Gibson (1975). While, *Phytophthora infestans* was identified according to the descriptions of Riberio (1978), Erwin *et al.* (1983), Ingram and Williams (1991) and Erwin and Riberio (1996).

Pathogenicity Tests

Pathogenic capabilities of the different isolated fungi were carried out using the detached leaf

technique under laboratory conditions and/or inoculating intact leaves under greenhouse conditions.

Inoculum preparation

Phytophthora infestans was grown on corn meal medium for 14 days at 18 ±2 °C in the dark. The fungal suspension was prepared by adding de-ionized distilled water to the culture and incubated at 5 - 8°C in refrigerator for 2 - 3 hrs to stimulate releasing of zoospores. The zoospore suspension was adjusted to be 8 x 10^4 zoospore/ml using haemocytometer technique (Brame and Lood, 1983).

Laboratory experiments

Apparently healthy leaflets on the fourth nodes were collected from 45 days old of Nicola potato variety. Detached leaves were placed in plastic trays 50 x 25 cm with moisten paper tissue sheets. The lower surfaces of the placed leaflets were inoculated separately with each of *P. infestans*, *A. solani* and *B. cinerea* spore suspensions (one ml/leaflet). Other sets of leaflets were inoculated with the same amount of distilled water only to serve as control. Moisture during experiment was maintained by covering trays with hyaline polyethylene sheet. All plastic trays were kept under room temperature (18±2°C). Inoculated leaflets were examined daily for 7-10 days for appearance of disease symptoms. Numbers of necrotic lesions as well as size of blighted area per leaflet were recorded and the % of the disease was determined using the disease index (7 categories) developed by Townsend and Heuberger (1943).

Symptoms category		Disease index
Zero	= no spots on the leaflet = no infection.	0
0-<5%	= few spots (1-3) on the leaflet = weak infection.	1
5-<10%	= (4-10) spots on the leaflet = moderate infection.	2
10-<15%	= (11-20) spots on the leaflet = moderate infection.	3
15-<25%	= 1/4 infected area on the leaflet = moderate infection.	4
25-<50%	= 1/2 infected area on the leaflet = severe infection.	5
50-<75%	= 3/4 infected area on the leaflet = severe infection.	6
75-100%	= more of leaflet area infected = very severe infection.	7

The percentage of the severity was calculated using the following formula:

$$\% \text{ Severity} = \frac{\text{sum of } n \times v \times 100}{5N}$$

Where,

n = number of leaves in each symptoms category.

v= numerical value of each category.

N= total number of leaves in sample.

Greenhouse experiments

Alternaria solani and *B. cinerea* were grown on Czapek Dox agar medium (Salam *et al.*, 2006) in plates 9 cm in diameter at 25°C for 7-10 days. The developed cultures were flooded by 5 ml sterilized water. The growth (spores and mycelium) were gently rubbed using smooth brush to remove fungal growth from the medium surface. The prepared suspension was received in sterilized flasks 250 ml. Obtained suspensions of spores and mycelium were filtrated through sterilized cheese cloth to reduce the mycelial fragments. Fungal suspension of each tested fungus separately was collected and adjusted using sterilized water to be 8 x 10^4 (*A. solani*) and 13 x 10^4 (*B. cinerea*) cfu/ml using haemocytometer technique. The prepared suspension of each pathogenic fungus was sprayed potato Nicola variety 45 days after planting in clay soil in plastic pots. Diseased plants and the percentage of disease incidence were calculated, compared with control treatment (sprayed with water only).

Varietal reactions

This experiment was carried out just to evaluate different potato varieties (Diamont, Nicola, Spunta and Valor) for their tolerance or susceptibility against different diseases under study. The evaluation of different potato varieties was carried out using the detached leaf technique under laboratory and/or intact leaves under greenhouse conditions.

Potato plants of the tested varieties were grown under greenhouse conditions. Potato plants were kept at 20±2 °C and high relative humidity (90%). Intact leaves were inoculated with each of *A. solani*, *B. cinerea* and *P. infestans* as described before. Another set of potato plants received only distilled water (control treatment). Only intact leaves were examined and disease symptoms seriously were assessed as mentioned before.

RESULTS AND DISCUSSION

Several fungal pathogens were obtained from infected leaves of potato collected from different

governorates. Identification of the obtained isolates was carried out at Mycology Department, Plant Pathology Research Institute, Agric. Res. Center, Giza. All of them were *Alternaria solani*, *Botrytis cinerea* and *Phytophthora infestans*. Pure isolates were maintained on PDA slants for further studies. The obtained results are in harmony with those recorded by Erwin *et al.* (1983), Ingram and Williams (1991), Heneriquez (2000), Steven (2005), Al-Maghrabi (2004) and Neelima and Ramunpeet (2004).

To determine the economic importance of potato foliage diseases caused by *Alternaria solani*, *Botrytis cinerea* and *Phytophthora infestans*, disease survey was carried out during 2004 growing season. Data in Table 1 show that all potato varieties were susceptible to infection by the three foliage diseases under the conditions of Sharkyia, Behera or Qalubyia governorates. Data also indicate that Behera governorate was the most favorable governorate for the appearance of all diseases where the highest disease incidence (64.14%) was recorded followed by Qalubyia governorate (60.71%) while, Sharkyia governorate occupied the third rank (55.56%). Data also indicate that there is sort of correlation between potato varieties and disease incidence in the same locations. Spunta variety showed the highest susceptibility to early blight caused by *A. solani* in Qalubyia and in Behera governorates (90%, 88.3%), respectively, while, Nicola was the most susceptible variety in Sharkyia governorate (81.7%). Gray mould was the highest disease incidence on Spunta variety in Sharkyia governorate (58.33%) followed by Nicola variety in Qalubyia governorate (61.7%) while, Diamont variety recorded the highest disease incidence in Behera governorate (65.7%). Late blight disease was showed the highest incidence in Diamont variety (73.3%) in Sharkyia governorate followed by Nicola variety in Qalubyia (65%) and Behera governorates and (80%).

Data obtained cleared that disease severity determined on the susceptible potato varieties was more severe during the season 2004. This may be attributed to the variable factors of the environmental conditions such as temperatures and relative humidity (fog dew and rains). The interaction between the

environmental conditions of any governorate and the genetic make up of tested varieties. These results are in agreement with Nachias *et al.* (1988), Fahim *et al.* (2003) and Waals *et al.* (2003). They attributed that the differences in disease severity were attributed to the variability of the cultivated varieties, the virulence of each of *A. solani*, *B. cinerea* and *P. infestans*, the applied cultural practices and the prevailed metrological factors, *i.e.* temperature and relative humidity (dew, clouds, fog and rains).

Pathogenic capabilities of 3 isolates belong to each of the three pathogens obtained from different governorates were studied. Data in Table 2 indicate that clear differences in pathogenicity were detected among the tested isolates. Pathogenic potentiality on Nicola variety determined as percentage of disease severity compared with control (whereas the percentage of disease incidence recorded 100%). The highest disease severity was observed when *A. solani* and *P. infestans* isolated from Sharkyia samples, being 35.2%, 45.63%, respectively. Regard gray mould disease, *B. cinerea* isolates varied in their reaction where Behera isolate showed the highest disease effect. The differences between the yielded lesions showed great variation among the various governorates. The pathogenic capability of the obtained pathogens of *A. solani*, *B. cinerea* and *P. infestans* were tested. Data obtained showed that the three pathogens were differed in their virulence due to their potentiality.

Sharkyia isolate of *A. solani* and *P. infestans* pathogens were the most pathogenic one, while Behera isolate of *B. cinerea* was the most pathogenic one. These results were confirmed with those mentioned by Mayton *et al.* (2001) for late blight, in case of early blight, Kuczynska (1983), Reifshneider *et al.* (1984), Kaul and Sexena (1988) for early blight, Rossal *et al.* (1980) and O' Neill *et al.* (1997) for gray mould. Differences in pathogenicity might be due to the differences among fungal isolates in their population, differences between potential hosts and host- parasitic reaction.

This work was carried out to investigate the susceptibility of different potato varieties against the tested pathogens. The reactions of four potato varieties against the tested three pathogens (*A. solani*, *B. cinerea* and *P. infestans*) were tested using the detached leaf technique. Data in Table 3 show

Table 1. Survey of different potato foliage diseases on different potato varieties at different localities, during 2004 growing season

Disease	Potato varieties	Disease incidence at different governorates				
		Sharkyia	Qalubyia	Behera	Means	L.S.D. 0.05
Early blight	Diamont	50	73.0	40.0	54.33	5.92
	Nicola	81.7	76.7	83.3	80.57	7.55
	Spunta	71.7	90.0	88.3	83.33	7.55
L.S.D. 0.05		7.88	8.415	7.55	-	-
Gray mould	Diamont	33.3	43.3	65.7	47.43	6.25
	Nicola	55.0	61.7	45.0	53.9	8.86
	Spounta	58.33	35.0	55.0	49.44	9.99
L.S.D. 0.05		8.86	9.25	6.9	-	-
Late blight	Diamont	73.3	51.7	50	58.3	4.62
	Nicola	35.0	65.0	80.0	60.0	10.34
	Spunta	41.7	50.0	70.0	53.9	9.99
L.S.D. 0.05		9.25	5.97	10.34	-	-
Means		55.56	60.71	64.14	-	-

Table 2. Pathogenicity test of three isolates of each of *Alternaria solani*, *Botrytis cinerea* and *Phytophthora infestans* on the susceptible potato (Nicola variety) plants under greenhouse conditions

Tested fungi and isolate source	%Disease severity	L.S.D. 0.05
Alternaria solani		
Sharkia isolate	35.2	3.96
Qalubia isolate	26.96	
Behira isolate	27.93	
Botrytis cinerea		
Sharkia isolate	23.6	4.17
Qalubia isolate	20.83	
Behira isolate	23.73	
Phytophthora infestans		
Sharkia isolate	45.63	5.7
Qalubia isolate	42.73	
Behira isolate	44.46	
Control	Zero	
Means	29.11	

Table 3. Susceptibility of different potato varieties to the three different foliage pathogens measured as disease severity using the detached leaf technique

Potato varieties	Tested fungi			Means
	Alternaria solani	*Botrytis cinerea*	*Phytophthora infestans*	
Diamont	62.08*	30.41	39.58	44.02
Nicola	39.17	29.18	79.17	49.17
Spunta	89.58	37.92	57.08	61.53
Valor	60.42	45.83	68.75	58.33
L.S.D. 0.05	17.69	16.72	10.47	

different levels of susceptibility of the tested varieties against the tested pathogens. Diamont and Spunta potato varieties were more susceptible to *A. solani* (62.08, 89.58%, respectively), compared with (39.17, 60.42%) for Nicola and Valor varieties. The latter was moderately susceptible to *B. cinerea* (45%). Valor and Nicola varieties showed the highest percentage of disease severity to *P. infestans* which recorded (68.75 and 79.17%) compared with Diamont and Spunta varieties (39.58 and 57.08%). Early blight, gray mould and late blight diseases caused by *A. solani*, *B. cinerea* and *P. infestans*, respectively are considered the most destructive diseases during production. The diseases have become endemic in the last few years in Egypt and affected the most cultivated varieties with different degrees due to their susceptibility. Four commercial varieties of potato were chosen to evaluate their reaction against *A. solani*, *B. cinerea* and *P. infestans*. Data obtained cleared that they were differed in their reaction against the aggressive isolate of *A. solani* and *P. infestans* from Sharkyia governorate and *B. cinerea* isolate from Behera governorate. These results could be expressed due to differences in the chemical and morphological structure of leaves. The former data are confirmed by several plant pathologists such as Vevetia Rodriguez *et al.* (2002) and Talvoja and Ojarand (2005). Also, the variability of varietal reaction among potato varieties might be attributed to the plant growth stage, cultivar and locality (Kuczynska 1992) and breading program Gonzales *et al.* (1998).

The conclusion that potato varieties are varying in their reaction against early blight disease was proved by Ismail *et al.* (1986), Lima *et al.* (1996) and Estevez *et al.* (1998). In late blight disease the differences between cultivars in their resistance and susceptibility might be due to the differences in their genetic make up which may affect on some morphological factors and host-pathogen relationships which play a role in cultivar susceptibility (Walker, 1975). Cultivar resistance might also be due to race specific (Goth *et al.*, 1983).

Determination of Disease Severity of the Three Pathogens on the Different Potato Varieties

Four different potato varieties were tested against the important potato foliage diseases under greenhouse conditions to find out the susceptibility of each. Data obtained in Table 4 show that disease severity differed according to the tested pathogen. The four tested potato varieties were susceptible to the three tested pathogens but at different degrees in disease severity. Nicola variety was the most susceptible for early blight caused by *A. solani* (26.33%) followed by Spunta variety (23.66%) while, the least variety was Valor (17%). On the other hand, potato varieties Spunta and Nicola were the most susceptible varieties to gray mould caused by *B. cinerea* (24.43% and 24.33%, respectively) while, the least one was Valor variety (12%). Nicola variety was the most susceptible variety to late blight disease caused by *P. infestans* (46%) followed by Spunta variety (35.33%) while, the least was Valor variety (31%).

Table 4. Percentage of disease severity on the different potato varieties foliage under greenhouse conditions

Potato varieties	Tested fungi			Means
	Alternaria solani	*Botrytis cinerea*	*Phytophthora infestans*	
Diamont	21.00	15.66	32.33	22.94
Nicola	26.33	24.33	46.00	32.22
Spunta	23.66	24.43	35.33	27.90
Valor	17.00	12.00	31.00	20.00
L.S.D. 0.05	5.23	5.83	10.93	

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دراسات على بعض أمراض المجموع الخضري في البطاطس

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تعتبر البطاطس من أهم محاصيل الخضر التابعة للفصيلة الباذنجانية حيث يصاب المجموع الخضرى للبطاطس بالعديد من المسببات المرضية خلال مراحل نموه المختلفة والتى تؤدى لخسارة كبيرة فى المحصول، ولعل أهم تلك الأمراض مرض الندوة المتأخرة التى يسببها الفطر فيتوفثرا إنفستانس، والندوة المبكرة التى يسببها الفطر ألترناريا سولانى، والعفن الرمادى الذى يسببه الفطر بوترايتس سينريا. لذا قامت هذه الدراسة بحصر الثلاثة أمراض المنتشرة فى بعض محافظات الدلتا (الشرقية – البحيرة – القليوبية). ولوحظ خلال دراسات الحصر على ثلاثة أصناف قابلة للإصابة (دايمونت – نيكولا – سبونتا) أن محافظة البحيرة كانت من أكثر المحافظات التى إنتشرت بها الأمراض الثلاثة السابقة. ومن خلال تجارب العزل من العينات المصابة تم الحصول على ثلاث عزلات من الفطريات الثلاث وقد تم تعريفها واختبار قدرتها المرضية إذ تبين أن لها القدرة على إحداث تلك الأمراض وذلك بتقدير شدة المرض. تم دراسة مدى قابلية أصناف البطاطس المختلفة للإصابة بالأمراض الثلاثة. باستخدام طريقة الورقة المنزوعة تحت ظروف المعمل والأوراق المتصلة بالنباتات تحت ظروف الصوبة. وقد بينت هذه الدراسة تباين الأصناف المختلفة للإصابة بالفطريات الثلاثة تحت كل من ظروف الصوبة والمعمل.