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STUDIES ON PYTHIUM LEAK ROT DISEASE ON POTATO TUBERS DURING STORAGE IN EGYPT.

Hanan A. El-Marzoky.
Agricultural Botany Department, Faculty of Agriculture, Suez Canal University.

ABSTRACT

Pythium leak is a post-harvest disease of potato tubers manifested by a wet, spongy rot that can quickly lead to complete tuber decay.Two isolates of *Pythium* sp. from Salhia and Ismailia districts were isolated from naturally infected potato tubers. The morphological characters examined were dimensions of oogonia ,oospores , antheridia , hyphal swelling and oospores wall thickness. Oogonia were mostly terminal and the hyphal swelling were mostly intercalary in the two isolates. These morphological characters of Salhia and Ismailia isolates were in accordance with the reference data of *Pythium ultimum var, ultimum*. . Pathogencity tests of the two *P.ultimum* isolates of Salhia and Ismailia revealed that Ismailia isolate capable to cause higher incidence of infected tubers and the depth of internal rot was more than that in case of Salhia isolate**.** Cultivar reactions indicated that Diamant cultivar was the most susceptible to both *P.ultimum* isolates, however, Maradona and Baraka cultivars were the least susceptible ones. In the same time, Spunta , King Edward, Alpha ,Desiree and Drage showed moderately reactions . The optimum temperature for growth of both isolates of *P.ultimum*growth on PDA was 30 ^{o}C . The lowest growth was observed at 5 and 40 ^{o}C. Laboratory screening tests with four fungicides indicated that Previcure-N inhibited completely at 150 ppm.Accorrding to the available literature knowledge; this is the first report of infection with leak rot of potato tubers caused by *P. ultimum* var*. ultimum* in Egypt.

Keywords :leak, decay,oogonia,aplerotic,hyphal swelling, intercalary.Corresponding author Email h_marzoki@yahoo.com

INTRODUCTION

Potato (*Solanum tuberosum*, L.) is the fourth most important food crop worldwide after wheat , maize and rice Gebhart and Valkonen,2001.The major diseases that plague potatoes in storage include pink rot, Pythium leak, late blight, dry rot, soft rot, silver scurf, black dot, and early blight. Leak is a post-harvest disease of potato tubers that can quickly lead to complete tuber decay. In North America*,* leak is most commonly caused by *Pythium ultimum var . ultimum* Trow Peters *et al*., 2005 .*Pythium*spp. are now considered as “fungus-like organisms” or “pseudo-fungi” and are placed in the Kingdom Chromista Kirk *et al*., 2008 .Typical symptoms include tissue and a dark gray to black discoloration of internal rotted tissues which have a spongy, wet texture and may contain cavities Salas and Secor 2001. Infections by the leak pathogen predominantly originate from cuts and wounds, and only occasionally occur through the stem end .This disease can cause severe losses in the field prior to harvest and after tubers are placed in storage facilities Lambert and Salas, 2001; Salas and Secor, 2001.

This pathogen is soil borne microorganism, which can survive in soil for a long time and attack a wide range of host plants. Wounding tubers occurring during cultural and harvesting operations increase the probability of infection. The objective of the present work was to isolate the causal organism from naturally infected potato tubers, prove the pathogenicity tests on potato tubers inoculated with the isolated pathogen isolates and evaluate cultivar reactions. Effect of different fungicidal concentrations and different temperature degrees on the radial growth of*P.ultiumm,in vitro*, was also studied.

MATERIAL AND METHODS

Isolation:

Stored Potato tubers showing typical symptoms of leak disease King Edward cv.were sampled and isolation of the semi fungus pathogen. The outer skin of infected tubers was removed and the internal diseased portions were transferred onto PDA medium supplemented with 200 mg/L Chloromphenicol Triki *et al* .,2001 and incubated at 25 ^{o}C for 5-7 days . Isolates of Salhia and Ismailia were cultured on PDA, hyphal tip used for purification of isolated pathogen .

Identification:

Identification of the isolates was carried out on cultures grown on potato dextrose agar supplemented with 200 mg / L Chloromphenicol. Isolates were identified according to their morphological characters (sexual organs, hyphal swelling, oospores, anthridia and oogonia). Measurements of 30 anthridia and oogonia were examined and recorded according to Waterhouse1967 and 1968 , Van der Plaats-Niterink, 1981 , Dick 1990 and Martin 1990&1992.

Pathogenicity:

Apparently healthy 12 potato tubers of cv. King Edward , uniform in size, were inoculated with each of the two tested isolates. Under aseptic conditions, inoculation was carried out , using cork borer . Cylindrical cores (5mm diameter x15mm thickness) were removed from disinfected surfaces of potato by dipping for 5 min in 1% sodium hypochlorite and rinsed in distilled sterilized water. Inoculums of *P. ultimum* were prepared by growing the isolated organism on PDA for 72 hours at 25^{o}C . Once *P.ultimum* colonized agar disc(5mm diameter) cut from the colony margin was placed into each hole and the core was replaced . Inoculated tubers were placed in plastic trays containing moistened paper and wrapped in plastic film to maintain a high relative humidity .Tubers were incubated at room temperature for four days. After incubation, tubers were sliced cross the inoculated holes. To evaluate leak rot (watery wound rot) , tubers were bisected longitudinally from the apical to basal ends .Tubers were split perpendicular to the longitudinal axis to assess leak .Tuber halves were covered with moistend paper towels to enhance the development of the watery blackish discoloration . Number of tubers showing symptoms of leak was recorded 30 min. after cutting. Incidence and percentage of leak rot was

calculated as follows :(numbers of diseased tubers / number of inoculated tubers) x 100. To determine leak severity, the maximum width (W, mm) and depth (D, mm) of discolored surface were recorded and the penetration of tissue (P, mm) were calculated using the formula described by **Lapwood *,et al*.,1984** : P = [W/2 + (D/4)] / 2 . Control tubers were inoculated with PDA disks.

Effect of temperature degrees on the pathogen growth*in vitro* :

To determine the optimum temperature degree for *P. ultimum* growth *in vitro*, 5mm diameter mycelium disks of ten days old PDA culture of the pathogen were transferred to the center of 90 mm Petri dishes containing PDA. Cultures were incubated at 5, 10, 15, 20, 25, 30,35or40^{o}C and colony diameter was recorded every day(mm/d) until hyphal tips reached the edge of the plates . Three replicates were used for each temperature degree.

Cultivar reactions:

Inoculations with *P. ultimum*:

Tubers were wounded at one side before inoculations. The wounding procedure involved the removal of periderm by manually abrading of 1 cm x 1 cm area with an abrasive near the middle of one side of each tuber according to Taylor *et al*. 2004. The abraded area had the periderm removed with little damage to the underlying tissue. Inoculation and incubation of inculated potato tubers were carried out as mentioned before . Control tubers were inoculated with PDA disc.

Disease Assessment

Inoculated tubers were removed from moistend chambers and were sliced in half through the point of inoculation. To evaluate leak rot, tubers were bisected longitudinally from the apical to basal ends to follow the characteristics of leak disease and disease incidence was determined as mentioned elier.

Laboratory evaluation of four fungicides on the growth of *Pythium* sp.:

Four fungicides namely Previcure -N, Ridomil gold plus, Ridomil gold MZ, Ridomil gold were tested *in vitro* to evaluate their effect on the growth of *Pythium* sp. The weight of each fungicide was calculated to give definit concentrations in parts per million (ppm) of its active ingredient (i.e.). Stock solutions and suspensions were prepared by adding the desired grams aseptically to the appropriate ml of sterile distilled water in conical flasks according to Fernando and Linderman 1994. The concentrations used were 50, 100, 150 and 200 ppm, and these were used for *in vitro* test as amendments on (PDA). The fungicides were filter-sterilized after stock solutions were prepared. The PDA was autoclaved and cooled to 45^{o}C before fungicide solutions were added. A graduated sterile syringe apparatus was used to add 10 ml of PDA to each 100 × 15 mm Petri plate. Then, 5-mm-diameter plugs were cut from actively growing colony margins of *P*ythium sp. and placed in the center of fungicide-amended medium in three replicate plates per treatment . The same fungicide-free medium , was served as control . Dishes were incubated at 25^{o}C in darkness for 7 days. Mycelial growth (colony diameter) was measured daily and mean of two colony diameters were taken at right angles to each other, minus the diameter of the

inoculum Wiswesser, 1976 and Cremlyn, 1980. The average radial measurements of the plates were taken. Percentage inhibitions of each of the fungicides at different concentrations were calculated using the formula by **Suleiman and Emua,2009 as follows:**

$$\% \text{ Inhibitions} = \frac{\text{Diam control plates} - \text{Diam in treated plates}}{\text{Diam control plates}} \times \frac{100}{1}$$

Statistical analysis:

The obtained data were statistically analyzed by analysis of variance (ANOVA) using the fisher LSD method. Means were separated by fisher's protected least significant differences (LSD) at P 0.05 level Gomez and Gomez, 1984.

RESULTS AND DISCUSSIONS

Symptomatology

Leak is a post-harvest disease of potato tubers manifested by a wet, spongy rot that can quickly lead to complete tuber decay. Disease begins when *P.ultimum* enters tubers through wounds occurred during planting, harvest, and handling operations. Symptoms of Pythium leak begin as tan water-soaked lesions around wounds on the periderm. Tissues swell around the wound and the periderm discolored and becomes moist. A dark distinct boundary between healthy and diseased tissue is apparent in infected tubers. Diseased tissues become watery and may contain cavities Salas and Secor 2001. When infected tubers are squeezed, dark, watery ooze is produced. When infected tissues were opened the affected areas show a gray-brown to black color and may have a pink tinge (Fig1). During storage, tubers totally rot within a week leaving only tuber shells with thin skins. Yield losses are directly related to tuber bruising and injury. Symptoms of tuber rots produced under natural and artificial infections were similar to those described by Peters *et al*., 2005 and Lui & Kushalappa, 2003 .

Isolation of the causal organisms:

Two isolates of *Pythium* sp. from Salhia and Ismailia districts were isolated from naturally infected potato tubers showing typical symptoms of Pythium leak disease . The two isolates were used for identification procedures.

Fig.(1): Typical symptoms of external (A) and internal (B) naturally infected King Edward potato tubers with leak disease caused by *Pythium ultimum* var *. ultimum* Trow, during storage.

Morphology of the pathogen and identification:

The morphological characters examined for identification were: dimensions of oogonia , oospores,antheridia , hyphal swelling and oospores wall thickness according to Sparrow1960 and Dick ,1990 .Percentages of terminal oogonia , monoclinous antheridia and intercalary hyphal swellings were also determined . Related data of *P. ultimum* Trow var. *ultimum* from Van der Plaats-Niterink ,1981;are presented in (Table 1&Fig.2and3).Terminal Oogonia of Salhia isolate reached 93% , globose, smooth-walled, sometimes intercalary ranged between 12-26 µm in diameter (average21.9 µm). While Ismailia isolate oogonia showed 89% , globose, smooth-walled, sometimes intercalary 11-25µm in diameter (average22.1µm). Oospores globose and aplerotic index(one each oogonia) ranged about 94 % for Salhia isolate,13-25µm in .diameter (average 21.2 µm) and 98 % for Ismailia isolate,15-24µm in .diameter (average 20.3 µm)(1-4 / oogonia). The thickness of oospores wall 1-2µm indiameter.(av 1.6 µm) for Salhia isolate and 0.9-1.3 um indiameter(av 1.4 µm) for Ismailia isolate . Anthridia were sac-like in form, mostly monoclinous in the two isolates. Monoclinousanthridia were less frequently observed in Ismailia isolate than Salhia one. The number of antheridia per oogonia was usually one in two isolates. The hyphal swelling were mostly intercalary in the two isolates . These morphological characters of Salhia and Ismailia isolates were in accordance with the reference data of *P. ultimumvar, ultimum* , except for thickness of oospores wall and the aplerotic index . accordance with the reference data of *P. ultimum var, ultimum* Van der Plaats-Niterink , 1981 , Dick ,1990, Triki *et al* 2001and Al-Sheikh &Abdelzaher 2010 .

Table 1: Morphological characteristics and dimensions of sexual organs and hyphal swellings in two isolates of *P. ultimum* var. *ultimum*.

Morphological organs*	**Salhia isolate**	**Ismailia isolate**	***P.ulimum* var. *ultimum*** **
Oogonia			
Position ; Terminal or Intercalary	Terminal (93%)	Terminal (89%)	Terminal , sometimes Intercalary
Shape	Globose	Globose	Globose
Surface	Smooth	Smooth	Smooth
Diameter µm***	12-26 av.(21.9)	11-25 av.(22.1)	14-24 av.(21.5)
Oospores			
Number	1	1-4	1
Shape	Globose	Globose	Globose
Aplerotic index(%)	94	98	Aplerotic
Oospore Diameter (µm)***	13-25 av.(21.2 µm)	15-24 av.(20.3 µm)	12-24 av. (18 µm)
Wall thickness (µm)***	1-2 av.(1.6)	0.9-1.3 av.(1.4)	Often 2 or more Thick
Antheridia			
Monoclinous **	93%	86%	Most monoclinous.
Number	1/ each oogonia	1/ each oogonia	1-3
Shape	Sac- like	Sac- like	Sac- like
Length(µm)***	7-16 av.(12.8 µm)	8-18 av.(12.1 µm)	-
Width(µm) ***	5-9 av. (7.5 µm)	4-11 av. (6.7 µm)	-
Diclinous****	7%	14%	Sometimes Diclinous
Hyphal swelling			
length(µm) ***	13-17.8	14-18.9	20-25(-29)
breath (µm) ***	8.6-10	9-11	<11
Intercalary hyphal swelling %	80.54	77.5	Intercalary,sometimes terminal globose

*** Each characteristic was examined using at least 30organs for two isolates.**
**** Data from Van der Plaats- Niterink 1981**
***** Number in parentheses are Means**
******(Anthridia):Monoclinous – branch off oogonial stalk , however, Diclinous – branch off separate hypha according to Waterhouse 1942 and Sparrow 1960.**

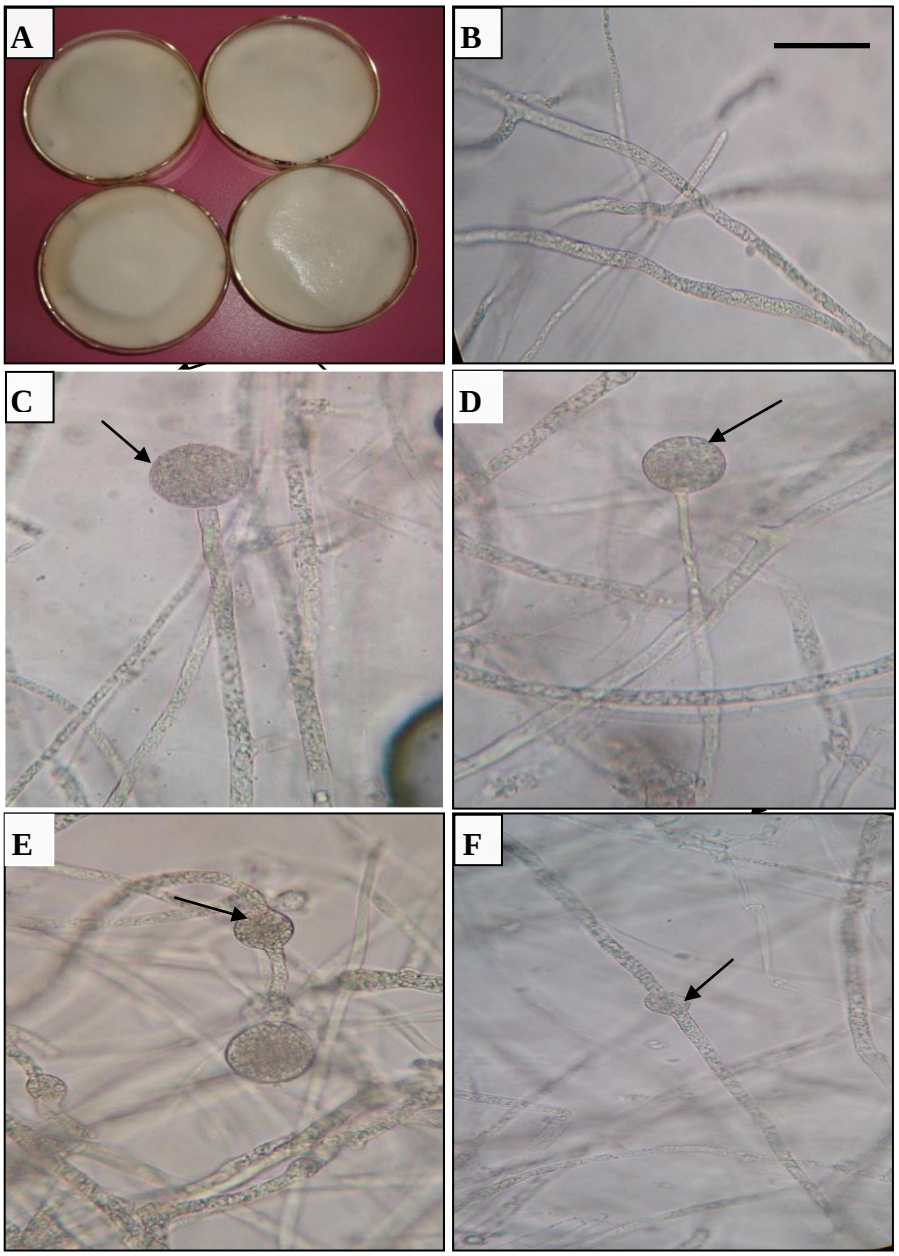

Fig.(2):Asexual organs of *P.ultimium* var *ultimum*: A. Colony morphology on PDA medium , B:mycelium nonseptate)cenocyticmycelium),C&D: Terminal hyphal swelling and E&F : Intercalary hyphal swelling.Bar 40µm

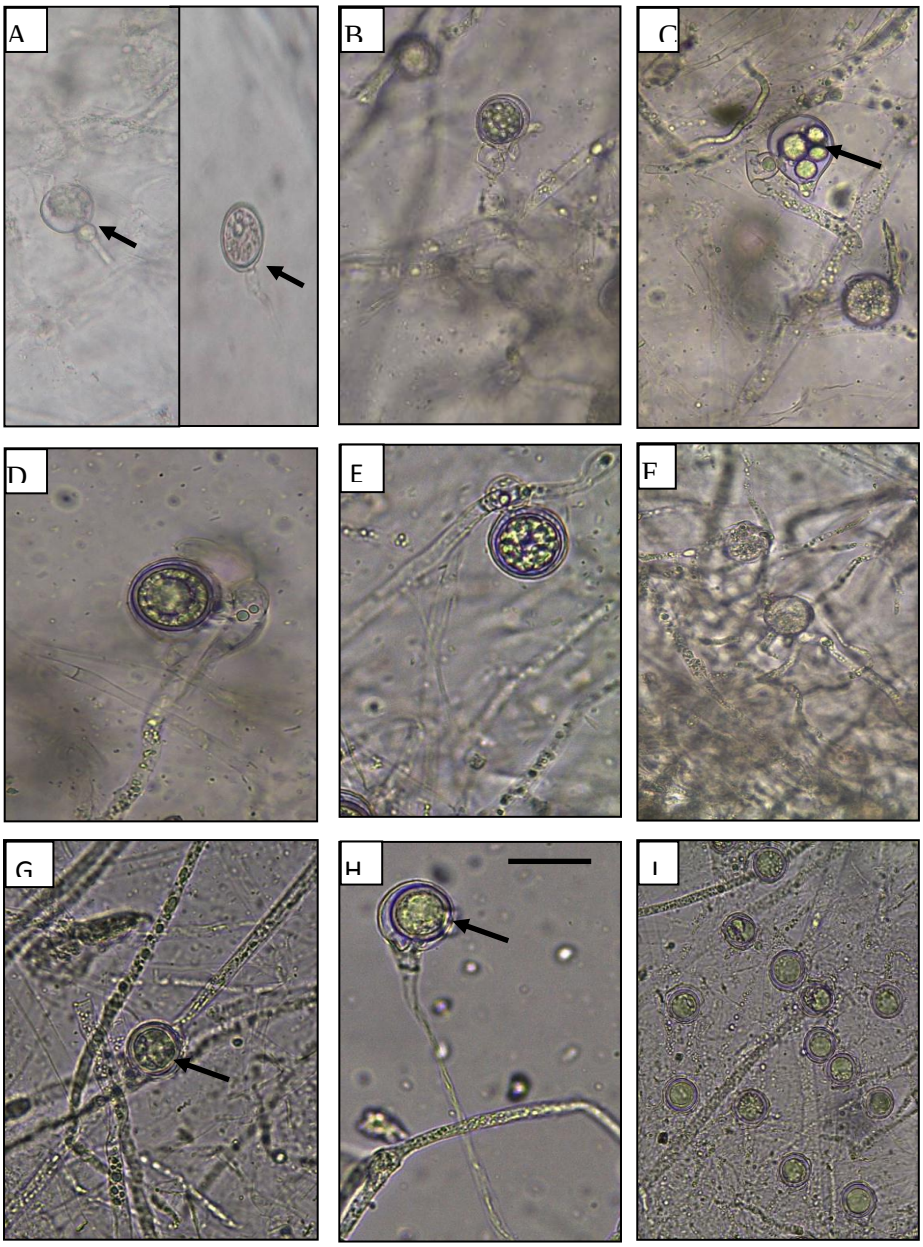

Fig.(3):Sexual organs :A: The oogonium with typical hypogynous anthridium ; B:Young oogonia with monoclinous ;C:Oogonium contaning four oospors with monoclinous antheridium;D,E and F:young oogonium with Diclinous;G:Intercalary plerotic oospore ;H: Teminal aplerotic oospore.,I: Abundant oospores of *P. altimum* on PDA .Bar 10 µm.

Pathogencity test (Koch,s Postulates) :

Pathogencity tests of two *P.ultimum* var *ulimum* isolates isolated during the present work from Salhia and Ismailia were tested on King Edward healthy potato tubers *in vitro* symptoms recorded 3 days after inoculation (Table2and Fig.4).Data showed that Ismailia isolate is capable for causing higher incidence of infected tubers (81. 33 %) and the depth of internal rot reached 44.6 mm . However, Salhia isolate showed moderate incidence of infected tubers (65.9 %) and the depth of internal rot reached 33.77mm. Inoculation with both *P. ultimum* isolates yielded typical symptoms of leak rot disease on all tested potato tubers within 3 days after inoculations . The obtained results indicate that isolates varied in their ability to incite the disease . Study to complete Koch's postulates confirmed that*P. ultimum* was re-isolated from diseased tissues and produced the same disease symptoms when isolates were subsequently re-inoculated into healthy tubers. This is in agreement with observations by Reen 1971 who noted a range of virulence among isolates of *Pythium ultimum* inoculated into tubers. This may favors oospore germinatnation and tuber leak development in tubers injured by insects and nematodes, as well as pathogen penetration into proliferated tuber lenticels or eyes. According to McKenzie & Lawrence 1981, watery wound rot (leak) may be found wherever potatoes are grown . To my knowledge , this is the first report of infection with leak rot of potato tubers caused by *P.ultimum* var*. ultimum* producing post harvest disease symptoms in Ismailia and Salhia districts Egypt.

Table (2): Pathogencity test of two *P. ultimum* isolates on King Edwerd potato tubers during April 2011 .

Isolates	**Incidence of infected tubers %**	**Depth of internal rot (mm)**
Salhia	65.9	33.77
Ismailia	81.33	44.65

Cultivar reactions:

This study was carried out on mature potato tubers of different cultivars including those commercially important in Egypt. Results indicate that these cultivars differed significantly in their susceptibility to both isolates , based on the incidence % and depth of internal rot (mm) . Diamant followed by Spunta , King Edward,Alpha ,Desiree and . Drage cultivars were the most susceptible to both *P.ultimum* isoltes , however, Maradona and Baraka cultivars were the least susceptible(Table3) . These results agree with those obtained by Priou *.et al*., 1997, they found different interaction between potato tuber cultivars and *P.ultimum* .On the other hand, Ismailia isolate was more pathogenic than Salhia isolate. The mean percentage of potato tuber rot incidence was higher with Ismailia isolate than that occurred with Salhia isolate. In the same time, non- significant differences were observed between the diameter of internal rot (mm) in potato tubers inoculated with Salhia and Ismailia isolates . Variations in reactions of these potato cultivars to the pathogen under study could be due to its chemical composition and nature of resistance in each cultivar.

Fig.(4). Artificially inoculated potato tubers incubatedat 25^{o}C , 3 days after inoculation with *P. ultimum var . ultimum* Trow, *in vitro* .

Table 3: Infection incidence (%) , depth and diameter of internal rot (mm) of leak rot disease caused by *Pythium ultimum* on inoculated tubers of 8 potato cultivars during 2010 season :

Cultivars name	Salhia isolate			Ismailia isolate		
	Incidence %	Depth (mm)	Diameter of internal rot (mm)	Incidence %	Depth (mm)	Diameter of internal rot (mm)
Diamant	61.6	38.9	36.4	59.3	42.6	41.3
Spunta	53.5	37.3	38.2	55.0	40.3	40.6
K. Edward	52.0	37.0	34.9	54.6	38.7	39.7
Alpha	27.8	35.8	35.1	44.1	37.9	37.8
Desiree	24.9	35.6	26.8	26.5	36.8	32.2
Draga	21.3	34.7	32.4	22.4	35.5	32.2
Maradona	19.3	34.5	3 0.3	20.8	35.0	30.2
Baraka	17.2	33.9	24.9	18.9	34.1	22.6
Total	277.6	287.7	259	301.6	300.9	276.6
Mean	34.7	35.9	32.4	38.2	37.6	31.2
LSD 5%	2.19	4.5	7.2	5.4	3.3	5.39

Growth of *P. ultimum* isolates on PDA at different temperature degrees ;

Effect of temperature degrees on growth of *P.ultimum* isolates on PDA, 24-hours after incubation at each temperature was studied . It appears from (Table 4) that the optimum temperature of *P.ultimum* isolates growth on PDA is 30 ^{o}C. Incubation at 20 - 30 ^{o}C favored greatly the *Pythium* growth. The lowest growth was observed at 5 ^{o}C, however, the lower growth was recorded at 10 and 40^{o}C, No clear difference was observed between Ismailia and Salhia isolates . All isolates were able to grow at 5-40 ^{o}C .The rate at the optimum temperature,30^{o}C was 36-38mm/24h.These results agree with those obtained byTriki *et al*.,2001 as they stated that 25-30^{o}Cwas the optimum temperatures for *P.ultimum* growth *in vitro* .

Table4:Rate of growth of *P.ultimum* isolates on PDA at different temperature degrees. Radial growth rate per 24hr (mm.)

Isolates	Radial growth rate per 24 hr (mm)							
	Temperature degrees							
	5^{o}C	10^{o}C	15^{o}C	20^{o}C	25^{o}C	30^{o}C	35^{o}C	40^{o}C
Salhia Isolate	4	8	12	25	30	38	24	8
Ismailia Isolate	3	9	11	24	29	36	22	6

Effects of fungicides on mycelial growth of *P.ultimum*isolates *in vitro* :

Laboratory screening tests with four fungicides were carried out to study their effect on growth of *P. ultimum*. Previcure - N , , Ridomil gold plus , Ridomil gold MZ and Ridomil gold were added to PDA medium at 50 ,100, 150 and 200 ppm and average diameter of colonies (mm) was recorded . It is noticed in Table 5 that the increase in fungicide concentration had resulted in an obvious increase in the inhibition of the linear growth of the tested *Pythium*. Prevecure N at 150 ppm completely inhibited the fungus growth .In the same time Ridomil Gold plus , Ridomil Gold MZ and Ridomil Gold completely inhibited the Pythium growth at 200 ppm .

Porter *et.al*., 2009 reported that several *Pythium* species causing leak on potato are managed by the systemic fungicide metalaxyl-M .The inhibitory effects of the fungicide showed certain level of significance at 0.05% compared with control . No significant difference was found between 50 and 100ppm. Similarly, fungicide concentrations as 100, 150 and 200ppm showed not significant differences on the *Pythium* growth. The effectiveness of Ridomil gold plus, Ridomil gold mancozeb and Ridomil gold increases with increasing the concentrations level. This observation agreed with the work reported by Lobna 2006.

Table(5):Effect of different concentrations of four fungicides on the inhibition percent of *P. ultimum* var *ultimum in vitro .*

Fungicides	Inhibition %			
	Concetration of fungicides –ppm.			
	50	100	150	200
Previcure- N	99.3	99.7	100	100
Ridomil gold plus metalxyl-M + copper(oxichloride)	98.5	99	99.7	100
Ridomil gold MZ (metalxyl+mancozeb)	98.2	98.7	99.3	100
Ridomil gold (metalxyl-M)	98	98.3	99.3	100
Control	0.0	0.0	0.0	0.0

LSD at 5% Fungicides (F): 5.16 - LSD at 5% conconcentration (C):1.09
LSD at 5% (F X C) : 1.99

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دراسات علي مرض عفن الرشح البيثيومي علي درنات البطاطس بعد الحصاد في مصر

حنان احمد المرزوقي

قسم النبات الزراعي - كلية الزراعة – جامعة قناة السويس

يعتبر مرض الرشح المتسبب عن الاصابة بالممرض البيضى*Pythium*يعد احد امراض ما بعد الحصاد التي يصيب درنات البطاطس. حيث يظهر الاعراض في صورة عفن مائي اسفنجي يؤدي الي التدهور السريع لدرنات البطاطس . تم عزل المسبب المرضي من كل من منطقة الاسماعيلية والصالحية من درنات البطاطس المصابة طبيعيا . تم فحص الصفات المورفولوجية للممرض البيضى وتشمل ابعاد اعضاء التأنيث والجراثيم واعضاء التذكير وانتفاخ الهيفات وسمك جدار الجراثيم البيضية .واتضح ان اغلب اعضاء التأنيث- الاووجونيا كانت طرفية في كلا العزلتين . وكانت الجراثيم البيضية التي لا تحتوي علي البرييلازم نسبتها تمثل ٩٤ % بالنسبة لعزلة الصالحية في حين تمثل ٩٨ % في عزلة الاسماعيلية .كما ان انتفاخ الهيفات يكون غالبا وسطيا داخل الهيفات وطبقا للمراجع تم تعريف الممرض بانه *Pythium ultimum* var. *ultimum* - تم اجراء تجارب العدوي الصناعية بكل من العزلتين علي درنات البطاطس السليمة – وتبين عزلة الاسماعلية كانت اقدر علي احداث الاصابة بدرنات البطاطس المحقونة وكان عمق العفن الداخلي بالدرنات كان اكثر من عزلة الصالحية .اوضحت نتائج مدي قابلية اصناف البطاطس المختبرة للعدوي بالمسبب المرضي مرتبة تنازليا كما يلي : دايمونت – سبونتا – كينج ادوارد - الفا- ديزرية - دراجا –مارادونا بينما كان الصنف بركة اقل الاصناف قابلية للاصابة . درجة الحرارة المثلي عند تنمية المسبب المرضي علي درجات حرارة مختلفة كانت ٣٠ درجة مئوية بينما اظهر الفطر نموا ضعيفا عند تنميته علي درجات حرارة ٥ او٤٠ درجة مئوية. أوضحت نتائج تقييم المبيدات علي النمو الفطري للمسبب ان المبيد بريفيكيور- ثبط نمو الممرض عند تركيز ١٠٠و١٥٠و٢٠٠ جزء في المليون . وطبقا لمعلوماتي المتاحة فان هذا اول تقرير يتضمن اصابة درنات البطاطس بعد الحصاد بمرض الرشح المتسبب عن شبيه الفطر *P. ultimum* var. *ultimum* في مصر .

قام بتحكيم البحث

أ.د/ محمد الششتاوى عبد ربة	**كلية الزراعة - جامعة المنصورة**
أ.د/ مصطفى حلمى مصطفى	**كلية الزراعة - جامعة عين شمس**