STUDIES ON *UROMYCES FABAE* (PERS.) DE BARY PRODUCED IN AXENIC CULTURE (UREDOSPORES GERMINABILITY, ENZYMATIC ACTIVITY AND PATHOGENICITY)

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ABSTRACT

Germinability of axenic uredospores of *U. fabae* isolates A and B was depended upon compsition of growth media. Percentage of germination was higher in uredospores of younger than older cultures and it was inversed proportionally with spore concentration. Germination was better in 2% than 4 or 6% of sucrose or glucose solutions in case of uredospores produced on MS-7 medium provided with extracts of broad bean leaves, meanwhile, uredospores formed on MS-7 medium alone germinated better in 4 and 6% glucose solution than the same concentrations of sucrose. Dikaryotic germ tubes, sprouted like yeast mycelium, oidiospores like-structures, germinated oidiospores, and infection structures i. e. appressoria, infection pegs, and infection hyphae were frequently observed during germination of uredospores.

Isolates A and B were quitely varied in their abilities to hydrolyzing starch and liquefying gelatin as well as in activities of pectulolytic and cellulolytic enzymes secreted in liquid growth media. The highest activities of pectulolytic and cellulolytic enzymes were obtained at pH values 6 and 6.6, respectively. The activity of constitutive and induced pectulolytic and cellulolytic enzymes were higher in isolate A than isolate B. However, the ratio between induced and constitutive pectulolytic and cellulolytic enzymes of isolate A and pectulolytic enzymes only in isolate B were narrower in filtrates of 14 and 21 compared with filtrates of 7 days old cultures. The opposite trend was noticed, however, in isolate B.

Under the *IN VITRO* conditions, the axenic uredospores of *Uromyces fabae* (Pers.) de Bary isolates A and B were able to infect both broad bean calli forming intercellular septate mycelium and oidiospores like structures and detached broad bean leaves forming oidiospores like structures in their epidermal tissues, however, under greenhouse conditions the typical rust pustule were frequently observed on inoculated leaves. Pathogenicity of iaolates A and B in relation to uredospore germination and activities of pectulolytic and cellulolytic enzymes was discussed. The isolate A produced few but larger concentric rust pustules surrounded by large numbers of minute pustules and clear yellowish halo zone meanwhile isolate B produced large numbers of smaller rust pustules without yellowish halo.

INTRODUCTION

Resting or germinating spores, usually uredospores, have provided the only material for metabolic studies on the free-living fungus (**Allen,** 1965, **Shaw,** 1964, **Staples** and **Wynn** 1965). As sporelings have failed to initiate saprophytic growth, most of these studies have been restricted to investigating biochemical and physiological events associated with spore germination and germ tube elongation.

However, if failure of the rusts to grow axenically were associated with a metabolic block in an essential pathway, it was hoped that these studies would reveal it.

Because the axenic culture of an obligate parasite is an interesting biological problem in itself and the present knowledge of the nutrition, metabolism, and physiology of the rusts has been restricted by the materials available for study, culturing the rusts are considered more important. In this regard, much work has been performed on the host-parasite complex (**Allen** 1954 & 1959; **Daly** 1967; **Hare** 1966; **Heitefuss** 1966; **Millerd** and **Scott** 1962; **Shaw** 1963 and **Yarwood** 1967) and significant changes have been observed in the metabolism of infected plants. However, it has not been possible to distinguish unequivocally between the relative contribution of host and pathogen to these changes. Some metabolic studies have been performed on mycelium isolated from infected host tissues. To obtain definitive data on the nutrition and metabolism of rusts it seems necessary to grow these organisms axenically. When we know how the parasite breathes, feeds, excretes and uses, within its host and at its host's expense, whatever biochemical processes link it to that host we may learn to control it. An exchange of nutrients, toxins, or both between the rust and host may determine whether the host is resistant or susceptible. Saprophytic cultures should prove useful in investigating these interactions, and so aid our understanding of mechanisms determining host resistance or susceptibility.

Uromyces fabae (Pers.) DeBary, the causal of broad bean rust disease, is one of the obligate parasites. There were no available literature about axenic culture of this pathogen. The present work was aimed to investigate uredospore germination, enzymes activities and pathogenicity of the resultant axenic uredospores.

MATERIAL & METHODS

Factos affecting urediospores germinability:

1-Effect of concentration of urediospores:

Uredospores of 14 days-old cultures of *U. fabae* isolates A and B grown on MS-7 medium alone or provided with broad bean extracts were used. Numbers of Uredospores /ml in each particular stock of urediospores suspension (extracted in 10 ml distilled water / plate) were counted. Stock spore suspensions were diluted to contain 80, 60, 40, and 20% of the counted urediospores. Few drops of each urediospore suspension from a particular dilution were placed on sterilized glass slides (12 slides fore each particular treatment), raised on U-shaped glass rods in Petri dishes lined with moistened filter papers then incubated for 6, 12, 18, and 24 hours at 25 C and 100% R.H. Percentages of germination of urediospores for each particular treatment were calculated.

2-Effect of growth medium and age of cultures:

Germinability of urediospores of the two isolates of *U. fabae* produced on 5, 10, 15 and 20 days old cultures grown on MS-7 medium alone or supplemented with broad bean leaf extract was tested as above mentioned.

3-Effect of concentrations of sucrose and glucose:

Urediospores produced from 5 days old cultures of the two isolates of *U. fabae* grown on MS-7 medium with or without broad bean leaf extract were used. Percentage of uredospore germinabilities in 0, 2, 4, and 6% sucrose or glucose solutions were determined as described above.

Starch hydrolysis and Gelatin liquefaction:

1-Hydrolysis of starch (Amylase test):

Uromyces faba isolates A and B were grown on nutrient agar-medium containing 0.2% soluble starch; beaf extract, 0.3%; peptone, 0.5%; agar, 1.5%; and distilled water, 1000 ml (Taha, 1964). The medium was sterilized as usual. The plates were inoculated with equal discs (6 mm diam.) of fungal growths taken from 7 days old cultures (grown on MS-7 medium) and incubated for different intervals at 25 °C. The resultant fungal growths were flooded with lugol's iodine. The presence of clear zone outside the area of the growth, indicating the extent of starch hydrolysis was measured and recorded.

2-Gelatin liquefaction: (Gelatinase test):

Equal discs of *U. fabae* isolates A and B were transferred from 7 days old cultures (grown on MS-7 medium) to plates of nutrient agar containing 0.4% of gelatin. The growth was flooded with 8-10 ml of solution of 15 g Hg Cl2. This reagent forms a white opaque precipitate with native gelatin but a liquefied is surrounded by a clear zone (Smith et al, 1952) which was measured and recorded as indicator the ability of these fungal isolates to liquefy gelatin.

Pectulolytic and cellulolytic enzymes activities:

Isolates A and B of *U. fabae* were left to grow for different periods at 25 C on liquid MS-7 medium (20 ml per each 100 cc flask) contained sucrose, 1.0% of pure citrus pectin or carboxsy methyl cellulose (CMC) as carbon sources. Five replicates for each particular treatment were used. The resultant fungal growths after each period were filtered through 3 layers of cheesecloth. The supernatant filtrates were frozen. until used for determination of pectulolytic (PG) and cellulolytic (Cx) enzymes activities by using the method described by Alexander (1954) as follow:

1-Effect of pH value on pectulolytic and cellulolytic enzymes activities:

In this study filtrates of 14 days-old liquid cultures of isolates A and B grown on MS-7 medium containing 1.0% of pure citrus pectin or carbosy methyl cellulose (CMC) as carbon sources were used. Effect of different pH values i.e.4.0, 4.6, 5.2, 5.6, 6/2, 6.8, 7.4 and 8.0 on polygalacturonase (PG) and cellulase (Cx) enzymes activities of both isolates was determined by following the change in viscosity of l.2% pure citrus pectin or CMC, respectively. Acetate buffer solution (0.1 M) was used for the first four pH values, while phosphate buffer solution (0.1 M) was used for the last four values. The reduction in viscosity was determined in the reaction mixture after 4 hours at 30 ?C. Enzyme activities were expressed as percentage of reduction in viscosity of the reaction mixture.

2-Effect of reaction time on activities of pectulolytic ancellulolytic enzymes:

Uromyces fabae isolates A and B were grown at 25 C for 21 days on a liquid MS-7 medium containing 1.0% pure citrus pectin or carbosy methyl cellulose (CMC) as sole sources of carbon then their filtrates were obtained. The obtained filtrates were used for determination of Pectulolytic (PG) and cellulolytic (Cx) enzymes activities expressed as percentage of reduction in viscosity of the reaction mixture as affected by time reaction (after 5, 10, 30, 50, 80, 120, 180 and 240 minutes) by using the method recorded by Alexander (1954). The pH values 6.2 and 6.8 were used for determining PG and Cx enzymes activities, respectively.

3-Effect of cultural age and substrate reaction on PG and Cx enzymes activities:

U. fabae isolates A and B were growfor 7, 14 and 21 days at 22C on a liquid MS-7 medium containing sucrose, citrus pectin and carboxy methyl cellulose (CMC) as sole sources of carbon. Fungal filtrates were then obtained and used for determination of both constitutive and induced pectulolytuc (PG) and cellulolytic Cx) enzymes activities by measuring % losses in viscosity of the reaction mixture. The above mentioned favorable pH values were used.

Pathpgenicity tests:

Both isolates A and B of *U. fabae* were left to grow for 5 days at 22 C on solid MS-7 medium with extracts of brad bean leaves. Urediospores of each isolate were obtained in sterilized distilled water. In each spore suspension the number of urediospores / ml was adjusted to 2000 urediospores / ml and used immediately in the following pathogenicity tests.

1-On host callus tissues:

Cleaned seeds of broad bean cv. Giza 402 were surface sterilized in an antiseptic solution (10-50% solution of commercial Clorox) for about 5 min., rinsed several times in sterile distilled water to remove any remaining traces of the disinfectant and allowed to germinate on a nutrient medium for 3-5 days. The healthy germinated seeds were transferred to Ms-medium in which thiamine.HCl has been increased to 0.2 mg/L as recommended by **Linsmaier** and **Skoog** (1965) and incubated for 30 day on 23C. The resultant calli were aseptically inoculated with urediospore suspensions prepared as above mentioned then incubated at 25 C and observed daily for up to 21 days. These calli were then killed and fixed in F.A.A. solution for 24-48 hrs, washed in 50% ethyl alchohol, dehydrated in series of ethyl alcohol 70, 90, 95 and 100%, infiltrated in xylene, embedded in paraffin wax with a melting point of 60-63C, sectioned of 15-20 microns in thickness, stained with the double stain method (malachite green and foxcen acid), cleared in xylene and mounted in Canada-balsam. Sections were microscopically examined to detect histological manifestations of noticeable responses resulted from pathogenicity.

2- On detached broad bean leaves:

Healthy leaves were detached from 30-40 days-old plants of broad bean cv. Giza 402, surface sterilized, thoroughly washed in sterilized distilled water, dried between sterilized filter papers, gently rubbed with fingers, sprayed with the above mentioned prepared uredospore suspension and placed at 100 R.H. inside Petri-dishes which were kept under room conditions (approx. 20 °C.) and examined daily for 7 days. Epidermal strips of inoculated leaves were mounted in drops of water and microscopically examined to detect manifestations of noticeable responses resulted from pathogenicity.

3-On plants grown under greenhouse conditions:

Thirty days old plants of broad bean (Giza 402 cv.) grown in sterilized soil in the greenhouse were used in this study. The two primary leaves were inoculated with suspensions of *U. fabae* -urediospores isolates A and B prepared as before mentioned. Inoculated plants were incubated for 24 hours in completely dark moist chamber with a water saturated atmosphere then transferred to normal greenhouse conditions. After 15 days the opened pustules were observed and photographed.

RESULTS

Factos affecting uredospores germinability:

1-Effect of concentration of uredospores:

It is clear from the obtained results (**Table** 1) that *U. fabae*-uredospores production was greatly enhanced on the modified MS-7 medium supplemented with broad bean leaf extracts compared with MS-7 medium alone particularly in case of isolate B which produced few numbers of uredospores when grown on the latter medium.

Table (1): Effect of concentration of uredospores of *U. fabae* **isolates A** and B grown on MS-7 medium alone or provided with broad bean extracts on % spore germination after different periods (hours).

MS-7 only					MS-7 with broad leaf extract				
Spores / ml	6 hrs	12 hrs	18 hrs	24 hrs	Spores / ml	6 hrs	12 hrs	18 hrs	24 hrs
Isolate A									
6760	4.0	7.7	8.7	8.7	11000	3.3	4.3	5.7	7.0
5408	10.0	13.0	16.0	22.3	8800	9.0	9.7	11.0	11.0
4056	11.3	15.0	17.0	20.7	6600	12.0	12.7	14.0	15.0
2704	11.7	14.7	17.7	24.0	4400	13.3	14.7	15.3	16.3
1352	17.3	18.0	24.0	28.7	2200	15.7	16.7	17.0	17.3
Isolate B									
330	6.66	11.00	11.3	23.00	3600	24.66	34.33	41.66	42.00
- *	-	-	-	-	2880	37.33	43.66	52.33	53.33
-	-	-	-	-	2160	45.33	50.33	54.66	55.00
-	-	-	-	-	1440	40.0	47.33	53.0	54.66
-	-	-	-	-	720	45.33	49.66	54.33	55.33

(-) = Not tested for poor uredospores formation.

Regardless kind of MS-7 medium and *U. fabae* isolate, percentage of germination of uredospores was inversed proportionally with spore concentration. It was conspicuously increased as uredospores concentration was decreased. It was higher in case of uredospores produced on MS-7 medium alone than those produced on the same medium provided with broad bean leaf extract, however the reverse order was noticed in case of isolate B. In most cases percentage of uredospores germinated was increased with time elapsed up to 24 hours from incubation.

2-Effect of age of cultures and composition of growth medium:

The data in Table (2) indicated clearly that percentages of *U. fabae*-uredospores germination were sharply decreased with increasing age of axenic cultures. The highest % uredospores germination was associated with uredospores of 5 dyas old cultures then decreased with aging of cultures up to 20 days particularly in case of uredospores produced from cultures grown on MS-7 medium with broad bean leaf extract. At different ages of cultures, % spore germination was higher in isolate B than isolate A.

Table (2): Effect of age of cultures of *U. fabae* isolate A and B on percentage of germination of uredospores (Uredospores suspensions adjusted to be contained approximately 2000 uredospores / ml):

	Isolate A grown on		Isolate B grown on	
Age (days)	MS-7 alone	MS-7 with leaf extract	MS-7 alone	MS-7 with leaf extract
5	18.3	42.3	-	69.3
10	17.3	31.7	-	59.3

15	17.0	24.0	-	54.7
20	13.3	11.7	-	28.0

3-Effect of different concentrations of sucrose and glucose:

From data in Table (3) it could be noticed that the highest percentage of uredospores germination were obtained when 2% sucrose glucose solutions were used as substrate for germination. Germinability of uredospores of both isolates A and B was slightly decreased with increasing concentration of both sucrose and glucose up to 6% particularly in case of uredospores produced on MS-7medium containing broad bean extract. In contrast, percentage of germination of uredospores formed on MS-7 medium without broad bean extract, was higher on 4 and 6% glucose solution than the same concentrations of sucrose.

Table (3): Effect of different concentrations of sucrose and glucose on percentages of germination of uredospores of isolates A and B produced on MS-7 with or without broad bean leaf extract..

Concen. %	Isolate A				Isolate B			
	MS-7		MS-7 with leaf extract		MS-7		MS-7 with leaf extract	
	Sucrose	Glucose	Sucrose	Glucose	Sucrose	Glucose	Sucrose	Glucose
0	42.3	42.0	18.3	18.0	--	--	69.0	68.0
2	44.0	43.0	25.0	18.3	--	--	77.3	70.7
4	22.0	42.7	21.7	12.3	--	--	76.0	70.0
6	12.7	22.0	20.7	11.0	--	--	67.7	57.0

Concerning with patterns of uredospore germination, it was noticed that 1-3 germ tubes were produced from germinated uredospore. The germ tube was divided to several cells by transverse septa. Each cell contained tow nucleii seen at opposite direction i.e. one at each side (Fig. 19). In distilled water, germ-tubes in isolate A produced lateral branches mustly at right angles while in isolate B it was mostly unbranched (Figs. 20 & 21). In presence of distilled water, the fragments of mycelium and germ tubes of germinated urediospores of isolate A changed to yeast like-mycelium. Cells of the latter mycelium were separated giving large numbers of one-celled oidiospores like-structures. (Fig. 22, a, b & c). It is interest to state that similar structures were noticed in the epidermal strips of broad bean leaves as well as in tissues of calli especially those inoculated by urediospores from axenic cultures of isolates A of *U. fabae* (Figs,). Some of these may be soonly re-germinated especially in presence of sucrose solutions producing strong septate mycelium. The formation of infection structures i. e. appressoria (Fig. 24a), infection pegs (Fig. 24b), and infection hyphae (Fig. 24c) were greatly stimulated in vitro by glucose or sucrose solutions.

Enzymatic studies on solid media:

1-Hydrolysis of starch (Amylase test):

Both isolates A and B can hydrolize starch and grow on a medium containing starch as sole source of carbon (Fig. 25). The ability of isolate A in secreting starch hydrolytic enzymes is faster than isolate B. Regarding halo-zone surrounded the resultant linear growth after treating with iodine, the obtained results (Table 4) indicated that starch hydrolytic enzymes activity expressed in term of

percentage of halo-zone was slightly decreased with aging of culture in case of isolate A, however the opposite trend was observed in case of isolate B.

Table (4): Linear growth of *U. fabae* isolates A and B and its surrounded halo zones on starchy medium flooded with iodine (Amylase test).

Culture age "in days"	Measurements of isolate A "mm)			Measurements of isolate B "mm)		
	Halo	growth	Halo %*	Halo	growth	Halo %*
3	25.0	8.0	68.0	6.0	6.0	0.0
6	29.0	10.0	65.0	8.0	7.0	12.5
9	45.0	18.0	60.0	10.0	8.0	20.0

Halo % = Surrounded halo-zone " mm" – Linear growth "mm" / Surrounded halo-zone " mm" x 100

2-Gelatin liquefaction: (Gelatinase test):

Table (5): Linear growth of *U. fabae* isolates A and B and its surrounded halo zones on gelatin-medium (gelatin liquifaction).

Culture age "in days"	Measurements of isolate A "mm)			Measurements of isolate B "mm)		
	Halo	growth	Halo %*	Halo	growth	Halo %*
3	31	9	71.0	13	7	46.2
6	40	12	70.0	18	8	55.6
9	45	15	66.7	26	9	65.4

Halo % = Surrounded halo-zone " mm" – Linear growth "mm" / Surrounded halo-zone " mm" x 100

In this experiment the abilities of both isolates A and B of *U. fabae* to liquefy gelatin (gelatinase test) were investigated. For verifying the surrounded halo-zone, the growth in plates was flooded with 8-10 ml of solution of 15 g Hg Cl2. The obtained data in Table (5) and Fig. (26) shows similar trend as that observed in the above experiment (Amylase test).

Enzymatic studies on liquid media:

1-Effect of pH value on pectulolytic and cellulolytic enzymes activities:

Uromyces fabae isolates A and B were grown at 25 C for 14days a liquid MS-7 medium containing 1.0% pure citrus pectin or carbosy methyl cellulose (CMC) as sole sources of carbon then their filtrates were obtained. Pectulolytic and cellulolytic enzymes activities in these filtrates as affected by different pH values expressed as percentage of reduction in viscosity of the reaction mixture were determined. The obtained data are in Table (6).

Regardless pH values, the obtained results (Table 6) proved that the induced activities of both pectulolytic and cellulolytic enzymes were higher in *U. fabae* isolate A than isolate B. With regard to pH values, it could be noticed that activities of these enzymes were slightly increased by increasing pH values in substrate reaction from 4.0 to 6.0 for pectulolytic enzymes and from 4.o to 6,6 for cellulolytic enzymes in both isolates A and B of *U. fabae* then decreased again by raising pH values above these limits. From these results it could concluded that the pH values 6.0 and 6.6 were the best for highest activitiese of pectulolytic and cellulolytic enzymes, respectively.

Table (6): Effect of pH value in reaction mixture on activities of pectulolytic and cellulolytic enzymes in filtrates of 14 days old cultures of *U. fabae* isolates A and B.

pH value	% reduction in viscosity of enzymes substrates			
	Pectulolytic enzymes activity of		Cellulolytic enzymes activity of	
	Isolate A	Isolate B	Isolate A	Isolate B
4.0	22.4	7.5	85.0	12.1
4.6	22.4	17.5	85.0	13.3
5.0	30.2	25.0	85.5	14.3
5.6	40.5	32.5	86.0	15.3
6.0	50.0	38.3	86.5	17.2
6.6	30.2	25.0	88.5	26.1
7.0	30.2	17.5	85.6	24.0
7.6	22.4	17.5	84.0	20.1
8.8	22.4	7.5	83.6	18.9
Mean	30.06	20.92	85.52	17.97

2-Effect of reaction time on activities of pectulolytic and cellulolytic enzymes:

The data in Table (7) stated that the activities of both pectulolytic and cellulolytic enzymes were steadily progressed by increasing time reaction from 5 to 240 minutes. The cellulolytic enzyme activity was very higher in isolate A after 5 minutes (51.7%) than isolate B (5.8%). After 240 minutes the lose in viscosity of substrate reaction reached to 42.9% and 80.9 in isolate A and 30.7% and 47.4& for pectulolytic and cellulolytic enzymes, respectively.

Table (7): Effect of time of reaction on activity progress of induced pectulolytic and cellulolytic enzymes in filtrates of 21 days old cultures of *U. fabae* isolates A and B:

Time In minutes	% reduction in viscosity of enzymes substrates			
	Pectulolytic enzymes activity of		Cellulolytic enzymes activity of	
	Isolate A	Isolate B	Isolate A	Isolate B
5	10.0	8.3	51.7	5.8
10	26.4	16.7	70.1	10.1
30	37.3	25.0	81.6	34.5
50	45.5	28.3	85.1	48.1
80	48.2	30.8	88.5	59.7
120	53.6	36.7	89.7	71.3
180	56.4	47.5	89.9	74.0
240	64.5	52.5	90.7	75.6
Mean	42.7	30.7	80.9	47.4

3-Effect of cultural age and substrate reaction on PECTULOLYTIC and Cellulolytic enzymes activities:

Uromyces fabae isolates A and B were grown at 25 C for 7, 14, and 21 days on the above mentioned MS-7 medium containing 3.0% sucrose, 1.0% pure citrus pectin or carbosy methyl cellulose (CMC) as sole sources of carbon. Constitutive and induced pectulolytic and cellulolytic enzymes activities in filtrates

of the resultant fungal growths expressed as percentage of reduction in viscosity of the reaction mixture were determined (Table 8). The proportions of induced to constitutive pectulolytic and cellulolytic enzymes activities in different old cultural filtrates of *U. fabae* isolates A and B were also calculated (Table 9).

Table (8): Effect of substrate reaction in growth medium * on pectulolytic and cellulolytic enzymes activities in 7, 14 and 21 days old fungal filtrates for isolates A and B of *U. fabae* .

Old "days"	% reduction in viscosity of enzymes substrates *							
	Constitutive enzymes				Induced enzymes			
	Pectulolytic		Cellulolytic		Pectulolytic		Cellulolytic	
	A	B	A	B	A	B	A	B
7	3.7	1.1	13.0	7.0	49.2	25.0	80.4	17.5
14	7.4	3.7	14.9	7.6	50.0	38.3	88.5	26.1
21	11.5	6.7	17.5	8.4	64.5	52.5	90.7	75.6
Mean	7.5	3.8	15.1	7.7	54.6	38.6	86.5	39.7

* MS-7 medium containing sucrose for constitutive pectulolytic and cellulolytic enzymes or citrus pectin or CMC for induced pectulolytic and cellulolytic enzymes, respectively.

Table (9): Ratio between induced and constitutive pectulolytic and cellulolytic enzymes activities in different old cultural filtrates of *U. fabae* isolates A and B.

Old "days"	*U. fabae* isolate A		*U. fabae* isolate B	
	Pectulolytic	Cellulolytic	Pectulolytic	Cellulolytic
7	13.3:1	6.2:1	22.7:1	2.5:1
14	6.8:1	5.9:1	10.4:1	3.4:1
21	5.6:1	5.2:1	7.8:1	9.0:1
Mean	8.6:1	5.8:1	13.6:1	5.1:1

The obtained results showed clearly that both pectulolytic and cellulolytic constitutive and induced enzymes were more active in filtrates of any given old culture of isolate A than isolate B (Table 31). Increasing age of cultures steadily increased the activity of both types of these enzymes. However, isolate B proved sudden increase in cellulolytic enzymes activity from 26.1 to 75.6% expressed as loss in viscosity of sreaction. The data in Table (9) which drived from those in Table (8) stated that, the ratios between activity of induced and constitutive pectulolytic enzymes of both isolates A and B were sharply decreased by in 14 and 21 days old cultural filtrates compared with filtrates of 7 days old cultures. Regarding with ratios between activity of induced and constitutive cellulolytic enzymes, the same trend was noticed also in case of isolate A only but reversed manner was associated with isolate B.

In Vitro Pathogenicity tests:

1- On host callus tissues:

On calli inoculated with axenic urediospores of isolate A of uromyces fabae, whitish mycelium was appeared after 5 days and completely covered callus tissue after 8 days from inoculation. After 21 days, the inoculated calli were shrink,

disintegrated and covered with brownish superficial mycelium with enormous numbers of urediospores. However, in case of isolate B, the whitish mycelium appeared after 15 days, covered about 50% of callus tissue and turned brown after 21 days from inoculation (Fig. 27). Tissues of infected calli showed intercellular septate hyphae after 10 days from inoculation with isolate A (Fig. 28 a). This intercellular mycelium became more dense and surrounded cell completely after 15 days (Fig. 28 b). After 21 days, inter- and intracellular oidiospores like structures were observed in infected tissues (Fig. 28 c, d & e). The latter structures were not observed in calli inoculated with isolate B. No haustoria like-structures were observed inside cells of inoculated calli.

2-On detached leaves:

Few urediospores could be germinate after 24-48 hours from inoculation with urediospores of Uromyces fabae isolates A or B. Long germ tubes with lateral branches penetrating leaves through stomata were observed on epidermal surfaces of the inoculated detached broad bean leaves (Fig. 29a). Microscopic examination of epidermal strips prepared from detached leaves after 7 days from inoculation with isolate A showed scattered groups of rounded cells similar to oidiospores which were mentioned before when urediospores allowed to germinate on glass slide in distilled water (Fig. 29b). Similar rounded cell were also observed in epidermal strips of naturally infected broad bean leaves (Fig. 29c). These groups of rounded cell may developed latter to produce the known typical rust pustules. No visible rust symptoms were observed, however, on these detached broad bean leaves even after 12 days from inoculation.

Pathogenicity test under greenhouse conditions:

In pathogenicity test carried out under greenhouse conditions typical rust symptoms due inoculation with urediospores produced in axenic cultures was clearly proved. The isolate A produced few but larger concentric rust pustules surrounded by large numbers of minute pustules. On broad bean leaves of Giza 402 cv. inoculated with isolate A yellowish halo zone was observed around the developed pustules (Fig. 30). In case of isolate B large numbers of smaller rust pustules without yellowish hall were observed on inoculated broad bean leaves of the same cultivar (Fig. 31).

دراسات على الفطر يوروميسز فابى النامي على المزارع الصناعية

من حيث إنبات الجراثيم اليوريدية والنشاط الإنزيمى والقدرة المرضية

عبدالمنعم إبراهيم إسماعيل الفقي – جهاد محمد دسوقى الهباء – خالد السيد عيد

كلية الزراعة بمشتهر – قسم النبات الزراعى

كان إنبات الجراثيم اليوريدية الناتجة من النمو الفطرى على بيئة موراشيجى وسكوج المعدلة بمفردها أعلى من مثيلتها الناتجة على نفس البيئة المزودة بمستخلص أوراق الفول وذلك فى حالة العزلة (أ) للفطر يوروميسز فابى، ومع ذلك فقد لوحظ العكس فى حالة العزلة (ب). وقد تناسب إنبات الجراثيم اليوريدية عكسيا مع كثافة الجراثيم ، كما حصل على أعلى نسبة إنبات للجراثيم اليوريدية على المزارع التى عمرها خمسة أيام ثم تناقصت النسبة تدريجيا حتى عشرون يوما. وكان أقل وأعلى تركيز من السكروز والجلوكوز لإنبات الجراثيم اليوريدية هو 2 و 6 % فى بيئة موراشيجى وسكوج المعدلة والمحتوية على مستخلص أوراق الفول. وقد أنتجت الجراثيم اليوريدية المنبتة من ا–3 أنبوبة إنبات ثنائية الأنوية ، كما لوحظ عند إنبات الجراثيم اليوريدية فى الماء تكون ميسليوم يشبه الخميرة وتراكيب تشبه الأوديات الجرثومية وقد لوحظ تكون تراكيب مشابهة لتلك التراكيب أسفل خلايا بشرة أوراق الفول وكذلك فى أنسجة الكالوس وخاصة الملقحة بجراثيم مأخوذة من المزارع الصناعية. وقد لوحظ بشكل متكرر وجود الأوديات الجرثومية المنبتة وكذلك تراكيب العدوى مثل الأبروسوريا وعضو الإختراق وهيفا العدوى عند إنبات الجراثيم اليوريدية فى محاليل الجلوكوز والسكروز. وكان نشاط الإنزيمات المحللة للسيليلوز والبولى جالاكتورينيز عاليا بالنسبة للعزلة (ا) عن العزلة (ب) وكانت درجة الحموضة المثلى لنشاط انزيم البولى جالاكتورينيز هى 6 فىحين كانت 6,6 للإنزيمات المحللة للسيليلوز. وقد تمكنت الجراثيم اليوريدية لكلا العزلتين (ا &

ب) الناميتين فى المزارع الصناعية من عدوى كالوس الفول البلدى. كما تطورت على أوراق الفول البلدى النامى تحت ظروف الصوبة بثرات صدأ مطابقة تماما لما هو فى الطبيعة ، حيث أنتجت العزلة (ا) بضع بثرات صدأ كبيرة الحجم ومحاطة فى نفس الوقت بعدد كبير من البثرات صغيرة الحجم ومنطقة إصفرار باهتة بوضوح حول البثرات بينما لوحظ أن العزلة (ب) تكون بثرات صغيرة الحجم كثيرة العدد بدون تكون لأى منطقة إصفرار باهتة.