

Table (3): Pectin methylesterase activity of virulent and avirulent isolates of P. solanacearum

Source of inoculum Isolates	*Milliliters of 0.01 N NaOH for neutralization of (-COOH) groups		
	Glucose nutrient broth	Glucose plus Calcium	nutrient broth
<u>Virulent:</u>			
Br1	6.8		4.1
Br2	6.6		4.8
Br3	7.9		3.2
Br4	11.2		8.6
Br5	10.8		5.8
Br6	11.7		5.5
Average	9.2		5.3
<u>Avirulent:</u>			
Br1A	5.3		4.8
Br2A	6.4		3.3
Br3A	4.2		3.2
Br4A	5.0		4.3
Br5A	4.2		1.5
Br6A	6.7		3.0
Average	5.3		3.4

\* as indication on PME activity.

anacearum. The activity was expressed as milliliters of 0.01N NaOH required for neutralization of (-COOH) groups after incubation for three hours.

The avirulent isolates under investigation showed lower PME activity as compared with virulent ones.

the recorded amounts of NaOH required for neutralization of liberated (-COOH) groups ranged from 4.2 ml to 6.7 ml for avirulent isolates with an average of 5.3 ml. The corresponding amounts for virulent ones ranged from 6.5 ml. to 11.7 ml. with an average of 9.2 ml.

Although incorporation of calcium in the propagation medium resulted in pronounced decrease in PME activity, the avirulent isolates showed lower activity of 3.4 ml as mean of all isolates, compared with virulent ones which was 5.3 ml. The latter was reported to be active in production of PME (Husain and Kelman, 1958; Wood, 1960; Shalaby, 1975; and Farag, 1976).

#### Cellulase Activity (Cx):

The activity of Cx as indicated by the loss in viscosity of carboxy methylcellulose (CMC) , is shown in Table (4).

Crude enzyme preparation (s) of avirulent isolates showed lower Cx activity as compared with virulent ones, either after 2 hours or 4 hours incubation at 30°C.

The recorded loss in viscosity produced by avirulent isolates ranged from 22.0% to 29.2% with an average of 25.5% after 2 hours incubation at 30°C. Virulent isolates, on the other hand, showed higher enzyme activity under the same conditions and the recorded loss in viscosity ranged from 28.1% to 38.4% with an average of 34.5%.

Further incubation of either avirulent or virulent isolates resulted in pronounced increase in activity that averaged 34.4% and 49.9%, respectively after four hours incubation at 30°C.

It is interesting to note that incorporation of calcium in the propagation medium resulted in sharp decline in Cx activity for both virulent and avirulent isolates under investigation. Although the deleterious effect of calcium on enzyme activity, the avirulent isolates showed lower activity (15.8%) as compared with virulent ones (16.3%) after 2 hours incubation at 30°C. Also the avirulent isolates showed lower activity (20.5%) as compared with virulent ones (31.2%) after 4 hours incubation at 30°C.

The virulent strains of P. solanacearum were reported to produce higher cellulase (Cx) activity (Husain and Kelman, 1958; Wood, 1967; Shalaby, 1975; Farag, 1976).

Table (3): Cellulase (Cx) activity of virulent and avirulent isolates of P. solanacearum.

Source of inoculum  Isolates	Glucose nutrient broth		Glucose nutrient broth plus calcium	
	% Reduction in viscosity after		% Reduction in viscosity after	
	2 hr.	4 hrs.	2 hrs.	4 hrs.
<u>Virulent:</u>				
Br1	35.6	48.1	27.2	36.1
Br2	38.4	53.0	29.0	30.5
Br3	38.4	50.3	28.6	32.7
Br4	28.1	49.2	28.8	30.5
Br5	33.2	53.8	23.4	28.8
Br6	33.5	45.2	20.8	28.5
Average	34.5	49.9	26.3	31.2
<u>Avirulent:</u>				
Br1A	22.3	38.0	15.4	18.0
Br2A	28.5	35.6	15.6	22.2
Br3A	28.5	29.2	15.0	20.5
Br4A	29.2	37.5	18.1	19.6
Br5A	22.0	33.0	16.8	20.5
Br6A	22.5	33.2	15.0	22.2
Average	25.5	34.4	15.6	20.5

The virulent strains of P. solanacearum were reported to produce higher cellulase (Cx) activity (Husain and Kelman, 1958; Wood, 1967; Shalaby, 1975; Farag, 1976).

Beta-glucosidase activity:

Sediment(s) of virulent and avirulent isolates of P. solanacearum did not show any B-glucosidase activity according to the method of Darweish and Wittenburry, (1970), as shown in table (5). The inability of the bacterium to produce B-glucosidase was also reported by Hayward, (1964).

Dehydrogenase Activity:

Data in table(6) showed the activity of glucose dehydrogenase for both virulent and avirulent isolates of P. solanacearum after 20 min incubation at 30°C.

The avirulent isolates showed higher dehydrogenase activity as compared with virulent ones.

The recorded activity of avirulent isolates ranged from 0.50 - 0.71 OD with a mean of 0.65 OD. While the corresponding range of virulent isolates was 0.20 - 0.34 OD with a mean of 0.25 OD.

The high dehydrogenase activity of avirulent mutants of P. solanacearum was reported by Kelman, (1954), to be higher than virulent ones.

Table (5): B-glucosidase activity of virulent and avirulent - isolates of P. solanacearum.

Density of black colour at 550 mμ			
Virulent Isolates		Avirulent Isolates	
Br1	00.0	Br1A	00.0
Br2	00.0	Br2A	00.0
Br3	00.0	Br3A	00.0
Br4	00.0	Br4A	00.0
Br5	00.0	Br5A	00.0
Br6	00.0	Br6A	00.0

**Table (6): Dehydrogenase activity of virulent and avirulent isolates of P. solanacearum.**

Optical Density at 640 mμ			
Virulent Isolates		Avirulent Isolates	
Br1	0.34	Br1A	0.50
Br2	0.30	Br2A	0.62
Br3	0.22	Br3A	0.71
Br4	0.22	Br4A	0.68
Br5	0.20	Br5A	0.68
Br6	0.20	Br6A	0.71
Mean	0.25		0.65

Effect of combined inoculation with virulent and avirulent strains on wilt development:

Table (7) shows the progress of wilt in stem inoculated cv. King Edward plants. Inoculation of virulent strains resulted in wilt expression after 35 days.

The recorded severities were 23.0%, 60.6%, 71.2%, and 83.6%, respectively after 35, 45, 55 and 65 days from inoculation.

Stem inoculation of avirulent strain, on the other hand, did not show wilt up to the end of the experiment. Root primordia on aerial stems and epinastic leaves were the major syndrome produced by avirulent strain.

It is interesting to note that combined inoculation of stems with virulent and avirulent strains (50% : 50%), delayed the disease onset and decreased wilt severities. The first incidence of wilt was recorded 45 days after inoculation and slightly increased up to the end of the experiment. The recorded severities were 15.8%, 25%, 30.3% after 45, 55, and 65 days respectively.

Data in table (8) show the progress of wilt on plants developed in infested soil. The onset of the disease started after 45 days from soil infestation. The recorded severities produced by virulent isolates were 22.5%, 38.2%, and 55.0%

Table (7): Effect of combined inoculation with virulent and avirulent strains on wilt severity (stem inoculation).

Treatments		Wilt Severity %			
		35 days	45 days	55 days	65 days
% virulent	% avirulent				
100	0	23.0	60.6	71.2	83.6
50	50	00.0	15.8	25.0	30.3
0	100	** 00.0	** 00.0	** 00.0	** 00.0

\*\* Root Primordia and leaf epinasty only.