

SURVIVAL OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* IN THE HOST TISSUE

BY

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Survival of the pathogen (*Xanthomonas campestris* pv. *campestris*) on seeds stored at different temperature revealed that infections survived in naturally infected seeds stored either at room temperature and lower temperature (10°C) for longer period (18 and 28 months respectively) than in seeds artificially inoculated with bacterial suspension (13 and 19 months respectively). With the passage of time there was a progressive fall in viability of the pathogen in the seeds which was very rapid after 8-9 months. Survival of *Xanthomonas campestris* pv. *campestris* in the stored infected stems and leaves of cabbage showed that survival under wet and dry storing conditions was virtually of the same magnitude initially, but with progress of time survival was more extended under wet conditions than in dry state. Similar results were obtained by carrying out tests on samples kept in open field as well as in the laboratory.

INTRODUCTION

Several workers concluded that the black rot disease caused by *Xanthomonas campestris* pv. *campestris* in epidemic form might occur from even small percentage of seeds carrying infection (Drechsler, 1919; Clayton, 1929 and Walker, 1941). It was the first bacterial disease found to be seed borne (Harding *et al.* 1904). Monteith (1921) suggested that the bacterium lodged on the seed coat during threshing and passed the winter in this location. Schaad and White (1974) found that in plots with remains of infected cabbage host tissue the bacterium could thrive upto 615 days if the number of viable cells on host tissue was about 10^5 per gram.

With the above findings as the back-ground experiments were conducted in order to investigate the pattern of survival of *Xanthomonas campestris* pv. *campestris* in host tissue under the tropical climate as prevailing in West Bengal, India.

MATERIAL AND METHODS

Survival on seeds stored at different temperature :

A study was undertaken to determine the survival potential of *Xanthomonas campestris* pv. *campestris* in naturally infected seeds lots stored at low temperature at $10^{\circ}\text{C}\pm 1^{\circ}\text{C}$ and room temperature ($28^{\circ}\text{C}\pm 4^{\circ}\text{C}$). Both the seeds of cabbage

(var. Eclipse Drumhead) and cauliflower (var. Snowball) were used for the study. Seed lots of more than 100 g each for cabbage and cauliflower obtained from commercial suppliers were stored. Seed sample containing 300 in each lot were then periodically tested for presence of *Xanthomonas campestris* pv. *campestris*. Seeds for this purpose, were washed 4 to 5 times with sterile distilled water, and then soaked for 12 hours in sterile distilled water. The soaked seeds were transferred inside sterilized petriplates containing two layers of moist blotting paper and kept inside BOD incubator at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 8 to 12 days. Each sample consisted of 300 seeds. Storing was done upto 28 months and samples for examination were drawn at an interval of one month. The number of seeds showing presence of pathogen was counted periodically. Data are presented as percentage of total count.

Observation to find out the presence of pathogen in seeds in 'Blotter' test were made. They consisted of observations on (a) plants showing rotting of cotyledons and hypocotyles appearing yellowish and pulpy colour (b) delayed germination in infected seed and stunted growth; (c) V-shaped lesion brown to black in colour on the margin of the infected cotyledons and true leaves. On cutting the infection portion with water, oozing of yellowish mass was observed under microscopic examination.

A similar experiment was undertaken with both cabbage (var. Eclipse Drumhead) and cauliflower (var. Snowball) where seeds were artificially inoculated. Seeds weighing 100g each were surface sterilized with 0.1% mercuric chloride solution and then repeatedly washed in sterile distilled water. They were then inoculated by thoroughly mixing them with the culture of *Xanthomonas campestris* pv. *campestris* (growth in Nutrient Agar medium for 7 days). The seeds mixed with bacterial culture then air dried and kept at room temperature ($28^{\circ}\text{C} \pm 4^{\circ}\text{C}$) for three days. Then they were stored in two different temperature stated before, namely room temperature and 10°C . Assay for the presence of bacterial infection was carried out and data were recorded in the same manner as described earlier.

Survival in naturally infected system and leaves :

Infected system and leaves were collected in lots from diseased plants in field in sufficient quantities. They were stored in two sets of large sized earthen pots containing normal soil and kept in open field. One such set of pots was regularly watered and the other set was left to the natural precipitation, if any, in the field. Isolation of *Xanthomonas campestris* pv. *campestris* was done periodically from these sample following standard procedures. Presence of the bacterium was confirmed by inoculation tests on healthy seedlings with the culture of the isolates from these sources. Isolation at 15 days intervals from 15th day stages was carried out. As a check, isolation was done prior to storing. Identical sets were

Table 1. Survivability of pathogen (*X. campestris* pv. *campestris*) on cabbage (*var. Eclipse Drumhead*) seeds
(No. of seed tested — 300 seeds in each case)

Days of observation	No. of infected seeds				Percentage of infection			
	Artificially inoculated		Naturally infected		Artificially inoculated		Naturally infected	
	Room temperature 28°±4°C	Lower temperature 10°C±1°C	Room temperature 28°±4°C	Lower temperature 10°C±1°C	Room temperature 28°±4°C	Lower temperature 10°C±1°C	Room temperature 28°±4°C	Lower temperature 10°C±4°C
Initial	300	300	36	36	100.00	100.00	12.00	12.00
30	248	267	36	36	82.86	89.00	12.00	12.00
60	227	224	36	36	75.66	74.66	12.00	12.00
90	180	209	34	35	60.00	69.00	11.33	11.66
120	183	158	32	35	61.00	62.66	10.66	11.66
150	169	162	32	33	56.33	54.00	10.66	11.00
180	155	158	30	30	51.66	52.66	10.00	10.00
210	138	147	29	28	46.00	49.00	9.66	9.33
240	131	142	26	28	43.66	47.33	8.66	9.33
270	83	127	21	27	27.66	42.33	7.00	9.00
300	77	124	18	25	25.66	41.33	6.00	8.33
330	61	120	18	25	20.33	40.00	6.00	8.33
360	48	113	16	21	16.00	37.33	5.33	7.00
390	42	114	12	21	14.00	38.00	4.00	7.00
420	Nil	109	9	18	—	36.33	3.00	6.00
450		91	8	18		30.33	2.66	6.00
480		79	6	15		26.33	2.00	5.00
510		65	6	12		21.33	2.00	4.00
540		61	3	12		20.33	1.00	4.00
570		34	Nil	10		11.33		3.33
600		Nil		10				3.33
630				7				2.33
660				5				1.66
690				3				1.00
720				3				1.00
750				2				0.66
780				2				0.66
810				2				0.66
840				2				0.66

Table 2. *Survivability of pathogen (X. campestris pv. campestris) on cauliflower (var. Snowball) seeds*
(No. of seed tested — 300 seeds in each case)

Days of observation	No. of infected seeds				Percentage of infection			
	Artificially inoculated		Naturally infected		Artificially inoculated		Naturally infected	
	Room temperature $28^{\circ}\pm 4^{\circ}\text{C}$	Lower temperature $10^{\circ}\text{C}\pm 1^{\circ}\text{C}$	Room temperature $28^{\circ}\pm 4^{\circ}\text{C}$	Lower temperature $10^{\circ}\text{C}\pm 1^{\circ}\text{C}$	Room temperature $28^{\circ}\pm 4^{\circ}\text{C}$	Lower temperature $10^{\circ}\text{C}\pm 1^{\circ}\text{C}$	Room temperature $28^{\circ}\pm 4^{\circ}\text{C}$	Lower temperature $10^{\circ}\text{C}\pm 4^{\circ}\text{C}$
Initial	300	300	29	29	100.00	100.00	9.66	9.66
30	236	244	29	29	78.66	81.33	9.66	9.66
60	199	227	29	29	66.33	75.66	9.33	9.66
90	171	181	28	28	57.00	60.33	9.33	9.33
120	152	156	25	28	50.66	52.00	8.33	9.33
150	134	134	21	28	44.66	44.66	7.00	9.33
180	118	131	20	26	39.33	43.66	6.66	8.66
210	110	125	18	24	36.66	41.66	6.00	8.00
240	120	121	18	24	34.00	40.33	6.00	8.00
270	91	116	17	23	30.33	38.66	5.66	7.66
300	73	105	15	21	27.66	35.00	5.00	7.00
330	66	94	11	21	22.00	31.33	3.66	7.00
360	55	72	11	19	18.33	24.00	3.66	6.33
390	47	61	9	19	15.66	20.33	3.00	6.33
420	38	50	7	18	12.66	16.66	2.33	6.00
450	23	46	7	15	7.33	15.33	2.33	5.00
480	17	40	7	12	5.66	13.33	2.33	4.00
510	Nil	40	4	10		13.33	1.33	3.33
540		34	4	8		11.33	1.33	2.66
570		25	4	5		8.33	1.33	1.66
600		14	2	5		4.66	0.66	1.66
630		Nil	2	3			0.66	1.00
660			2	3			0.66	1.00
690			Nil	2				0.66
720				2				0.66
750				2				0.66
780				Nil				

kept in the laboratory of which one was kept moist by regular watering and another without any addition of water.

RESULTS AND DISCUSSION

From the data presented in Tables 1 and 2, it would be seen that in both cabbage cauliflower, viable infection was noticed for a longer period in naturally infected seed lots than in the case of artificially inoculated ones. Infection was found to remain present under natural conditions upto 17 months in the former, while in the latter upto 13 months. In both the cases, seeds stored at lower temperature showed presence of viable infection for a much longer period. In case of naturally infected seeds, stored at 10 C, viable infection could be noticed till the end of the experiment, namely 28 months. From the initial stage, there was progressive decrease in the number of seeds showing presence of viable pathogen. After a period of eight to nine months (240 to 270 days) a somewhat rapid decline was recorded. Percentage of infection in naturally infected seed lots at any comparable stage was, however, much lower than in artificially inoculated ones.

Table 3. *Survivability of pathogen (X. campestris* pv. *campestris) on infected leaves and stems in open field*

Days of observation	Infected leaves		Infected stems	
	Dry condition	Wet condition	Dry condition	Wet condition
Initial	****	****	****	****
15	**	**	**	****
30	Nil	**	*	***
45		Nil	*	**
60				**
75				*
90				*
105				Nil

****=Very Heavy ***=Heavy **=Moderate *=Slight

Results regarding survival of the pathogen of infected leaves and stems left to the field soil and exposed to dry and wet conditions are presented in Table 3. It would appear from the observation recorded at intervals of 15 days that upto 15 days on leaves and stems pathogen was alive under both wet and dry condition. But storage under dry condition affected the survival of pathogen eliminating the bacterium totally by 30th day in infected leaves and by 45 days in infected stems. The survival of pathogen was much longer in the infected stem kept wet but on leaves kept under wet conditions which pathogen could be detected upto 45 days.

Similar study was conducted under storage condition in the laboratory and results are presented in Table 4. It would be seen from the Table 4 that survival of the bacterium was for much lesser period in infected leaves stored under dry condition

Table 4. *Survivability of pathogen (X. campestris pv. campestris) on infected leaves and stems stored in the laboratory*

Days of observation	Infected leaves		Infected stems	
	Dry condition	Wet condition	Dry condition	Wet condition
Initial	****	****	****	****
15	***	***	****	****
30	**	**	***	****
45	Nil	**	***	****
60		Nil	**	***
75			**	***
90			**	***
105			*	***
120			Nil	***
135				***
150				***
165				**
180				**
195				**
210				**
225				*
240				Nil

****=Very Heavy ***=Heavy **=Moderate *=Slight

(30 days) than under wet condition. The greater period of survival of the pathogen was in the infected stem than on the infected leaves. So far infected leaves were concerned, storage in the laboratory, under controlled condition did not increase the period of survival as compared to that in the field, but in infected stems under laboratory storage, survival was for a much longer period, upto 105 and 220 days in dry and wet condition, as compared to 30 and 45 days respectively.

Studies on survival of pathogen in the seeds, both under natural and inoculated conditions of infection through bacterization of the surface showed presence of pathogen for a longer time at low temperature than at room temperature. It was interesting to note that viable infection was present for much longer period in naturally infected seeds, than in artificially infected ones. This observation points out that under natural conditions infections is deep seated, and not superficial as would be achieved by bacterization on the surface. These findings suggest that treatment of seeds should be so designed as to get rid of deep seated infection.

Survival of *Xanthomonas campestris* pv. *campestris* in the infected stems and leaves is very important from practical point of view. Schaad and White (1974) mentioned perpetuation of the pathogen for a fairly long period in infected plant residues. Their observation was based on findings in temperate countries where average temperature being much lower more congenial conditions are present for perpetuation. Hence period of survival in this study was, upto 90 days, whereas Schaad and White (1974) reported 61 days. As would be expected, infected stems were found to be better sources for perpetuation and survival than the leaves which were more quickly affected by microbial competition in the soil.

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