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SURVIVAL OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* IN SOIL

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Studies to have information on the survival of *X, campestris* pv. *campestris* in different soil conditions showed longer period (35 days) of survival in moistend soil with higher (50%) moisture content than with lower moisture (10-25%) content (14 days) tn sterilized and non. sterilized conditions. In sterilized soil survival for a longer period (42 days) was observed than non-sterilized soil (35 days). Survivability tested on different soil *p*H showed that near neutral *p*H (6.8) was the best for the bacterial growth. Acidity, alkalinity and salinity affected adversely the survival, inhibiting effect of the last one being more pronounced.

INTRODUCTION

Xanthomonas campestris pv. *campestris* the causal agent of black rot disease attacks many cruciferous plants (Walker, 1941, Cook *et al.* 1952). Perpetuation of this organism pv. in soil was investigated by Schaad and White (1974). They noted that the bacterium could not be recovered 14 days or 42 days after removal of cabbage host from the fields in summer and winter respectively. They also worked out the half live of the bacterium in the soil to be 2.6, 1.3, 1.2 and 0.4 days during winter, late spring, early summer and late summer respectively.

It is a common knowledge that the survival of any microbe in soil is greatly influenced by the environment and microflora surrounding them. To study the possible role of such microflora or environment on the perpetuation of *Xanthomonas campestris* an experiment was undertaken in soils kept under different physical conditions.

MATERIALS AND METHODS

Role of soil moisture :

Pure culture of *Xanthomonas campestris* isolated from infected cabbage plants was grown in nutrient broth. Fifty millilitres of this broth culture after 7 days' growth of the bacterium was applied to 200 g of dry sterilized soil (*p*H 6.8) in glass beakers of 250 ml capacity. Soil moistures were maintained at 10%, 30%

and 50% level on air dry basis. Moisture of the beakers was maintained by periodic addition of sterile distilled water. Water holdiug capacity was determined following the procedure of Keen and Raczkowasci (1921) After application of the bacterial culture, beakers were kept inside BOD incubator regulated at 28°C+1°C. After a period of 72 hours, count of bacterial cells was made and the beakers were kept in room temperature (30 C+2°C). Soil samples were taken at weekly interval starting from 7 days of application of bacterial population. Dilution planting technique was adopted for this purpose. Three replications were taken for each moisture samples. Identification of the bacterium isolated was made in the usual way.

Survival in sterilized and non-sterilized soil :

Data of the previous experiment on survival of the bacterium in sterilized soil showed that the bacterium could live upto a limited period in the sterilized soil. This observation prompted to take up an experiment on comperative study of survivability in sterilized and non-sterilized soil.

In this experiment, soil samples (200 g each in 250 ml capacity beakers) were sterilized at 20 lbs pressure for 15 minutes in an autoclave and the moisture was regulated at 10% and 50% level on air dry basis. Cultures (50 ml broth) of *Xanthomonas campestris* pv. *campestris* were applied to such sterilized soil. An identical set was maintained with non-sterilized soil. The *p*H of soil in both sets was 6.8. Beakers were kept in BOD incubator at 28°+1°C for 3 days after which they were taken out and kept at room temperature. Data were recorded in the same manner as before.

Role of different soil *p*H :

In this experiment survival of *Xanthomonas campestris* pv. *compestris* was studied using different soil samples collected from different parts of the State (West Bengal, India) and having different *p*H (4.4, 6.8, 9.0 and 8.5) ranging from acidic to alkaline and saline. The *p*H was determined according to the method of Smiley and Cook (1972) using 0.01 N calcium chloride. The *p*H was measured in Electronic pH meter (Systronix) using a combined electrode. Inoculation of soil by culture of *Xanthomonas campestris* pv. *campestris* was done in the same way as in the previous experiment in 200 g sterilized soil in glass beakers of 250 ml capacity with moisture adjusted at 50% level. Beakers were kept at room temperature (30 C±2 C) and after 3 days of inoculation in BOD incubator at 28°C±1°C. Observations were recorded in the same way as in earlier experiments. Three replicatious were used for each *p*H level of soil studied. Survival of the bacterium was determined by dilution plating technique (upto 10^5 dilution) in nutrient agar medium. Initial bacterial populations

were counted after 72 hours of incubation in BOD at 28°C±1°.. Three replication were taken for each *p*H level.

RESULTS

Data presented in Table 1 clearly indicated that moisture level had a positive role in the survival of the bacterium *Xanthomonas campestris* in the soil. Higher the moisture content, greater was the period of survival. With increase in period of storage, there was a steady decline in the population which was most marked at 10% level, where the bacterium could not be isolated after two weeks of soil inoculation. At 20% level, its presence could not be noted after three weeks of storage, whereas at 50% level, the organism could be isolated upto five weeks. It may be partinent to point out that *Xanthomonas campestris* pv. *campestris* is not soil borne in the true sense of the term.

Results presented in Table 2 show that the bacterium could survive in unterilized soil upto five weeks, but at any comparable level, population was much less in the unsterilized soil. Effect of moisture was similar to that noted in Table 1. In this experiment it was observed that at 50% moisture level in sterilized soil, bacterium could survive upto 6 weeks but in unsterilized soil upto five weeks. Eflect of moisture was definitely pronounced and showed similar trends as in the previous experiment (Table 1).

Table 1. *Survivability of* X. campestris *pv.* campestris *in soil at different moisture levels*

Days of observation	Number of viable bacterial cells (X10^5)/g of dry soil (Average of three replicates)		
	Air dried 10% moisture levels	30% moisture level	50% moisture level
7	108.66	131.66	142.33
14	66.66	94.66	129.66
21	Nil	52.00	79.66
28	Nil	Nil	52.66
35	Nil	Nil	32.33
42	Nil	Nil	Nil

Initial No. of bacterial cell/g of dry soil at 10% moisture level = 155.33 x 10^5
Initial No. of bacterial cells/g of dry soil 30% moisture level = 149.66 x 10^5
Initial No. of bacterial cells/g of dry soil at 50% moisture level = 153.00 x 10^5

Data presented in Table 3 show the effect of *p*H on survival of the bacterium, was very pronounced. The bacterium survived best in near neutral *p*H—6.8, both acidity (*p*H 4.4) and alkalinity adversely affected the same. It was evident that under conditions of acidity, bacterium could survive for a comparatively

Table 2. *Survivability of* X. campestris *pv.* campestris on *sterilized and non-sterilized soil at different moisture levels*

Days of observation	Number of viable bacterial cells (X10⁵)/g of dry soil (Aberage of three replicates)			
	Sterilized soil		Non-sterilized soil	
	10% moisture level	50% moisture level	10% moisture level	50% moisture level
7	91.33	127.66	76.33	121.00
14	41.33	104.00	30.66	99.66
21	Nil	73.33	Nil	62.00
28	Nil	56.33	—	48.33
35	—	33.33	—	19.00
42	—	21.00	—	Nil
49	—	Nil	—	—

Initial number of bacterial cells/g in sterilized soil at 10% moisture level = 132.00 x 10⁵ & at 50% moisture level = 135.66 x 10⁵
Initial number of bacterial cells/g in non-sterilized soil at 10% moisture level = 127.66 x 10⁵ & at 50% moisture level = 130.33 x 10⁵

Table 3. *Survivability of* X. campestris *pv.* campestris *in soil at 50% moisture level at different pH*

Days of observation	Number of viable bacterial cells (X10⁵)/g of dry soil (*p*H Average of three replicates)			
	4.4	6.8	8.0	8.5
7	128.00	132.00	57.33	107.66
14	103.33	108 33	Nil	53.00
21	66.00	72.66	—	36.00
28	41.66	55.33	—	Nil
35	Nil	22.33	—	—
42	—	Nil	—	—

Initial number of bacterial cells/g of dry soil at *p*H 4.4 = 142.33 x 10⁵
Initial number of bacterial cells/g of dry soil at *p*H 6.8 = 145.33 x 10⁵
Initial number of bacterial cells/g of dry soil at *p*H 8.0 = 140.66 x 10⁵
Initial number of bacterial cells/g of dry soil at *p*H 8.5 = 147.00 x 10⁵

longer period than under alkaline conditions. Very limited survival (upto 7 days) a *p*H 8.0 was due to sal nity (4.18 m mohs/cm) rather than effect of *p*H.

DISCUSSION

Study in the perpetuation of the bacterium in both sterilized and unsterilized soil under near optimum condition (50% moisture level) showed that bacterium could survive upto a period of 5-6 weeks depending whether the soil was sterilized or not. Even a moisture of 30% was sufficient to sustain the microbe

for 3 weeks. These observation point out that though the bacterium is not soil borne, yet it can remain alive in soil for a limited period. According to Schaad and White (1974) the bacterium had such short half lives as 2.6 days in winter and 0.4 days during summer. From a practical point of view it may appear that in fields where there were outbreak of black rot disease in cabbago and cauliflower during winter leaving the field dry for a period to reduce or to eliminate the bacterium than by employing the same plots for immediate cropping.

Neutral *p*H around 6.8 was best for the survival of this bacterium. Neither highly acidic nor alkaline *p*H were found to be helpful. This to some extent, accounts for greater incidence of the disease in areas with soil ranging from pH 6 to 7.

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