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PATHOGENECITY TEST BY USING DIFFERENT INOCULATION METHODS ON *XANTHOMONAS CAMPESTRIS* PV *CAMPESTRIS* CAUSED OF BLACK ROT OF CABBAGE

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ABSTRACT

Black rot is a major disease of cabbage (*Brassica oleracea* var. *capitata*), caused by *Xanthomonas campestris* pv. *campestris (Xcc)* . Using six different inoculation techniques, such as (1) Spraying the inoculums by way of hand atomizer (2) Carborundum abrasion method (3) Multi-needle pricking method (4) Injection infiltration method (5) Hydathodes inoculation method and (6) Scissor clipping among these six techniques carborundum abrasion method gave quicker symptom expression as well as lesion progress on leaf , followed by the hydathode inoculation method, spray inoculation after multi-needle pricking and spray inoculation on uninjured surface respectively.

KEYWORDS: Pathogenecity Test, Inoculation Methods and *Xanthomonas campestris* pv. *Campestris*

INTRODUCTION

This disease was first described by botanist and entomologist Harrison Garman in Lexington, Kentucky, USA (1889). Since then, it has been found in nearly every country in which cabbage is commercially cultivated. Black rot is a bacterial disease that affects crucifers, particularly damaging the cabbage and cauliflower. But rape seed, mustard, turnip, kohlrabi, Chinese cabbage and many other members of cruciferous family are also found susceptible to the disease. In suitable conditions it may become serious and many growers sustain severe economic loss. The losses due to the disease may exceed 50% in warm, wet climates. In some cases, the crop may be a total loss. *X*. *campestris* pv. *citri* is a rod shaped, gram negative bacterium, with single polar, flagellum. Growth is obligatory aerobic, maximum temperature for growth is 35-39°C and the optimum temperature is 28-30°C. Bhat *et.al.*(2000) reported that black rot caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson was a serious disease of cabbage in Jammu and Kashmir. Amongst different inoculation methods tested, 'Vein inoculation' method gave quicker symptom expression and highest lesion progression followed by 'Hydathode inoculation' and 'Clip inoculation' methods respectively. Spray inoculation for stomatal penetration of the pathogen failed to express any symptoms. A suitable, convenient and ensured method of inoculation is very important for various field and green house studies including testing of varieties resistance. Bacteria enter leaves and stem mainly through stomata, lenticels and wounds. Among artificial methods of inoculation, spraying bacterial suspension under pressure on to a leaf surface without visible water soaking of the leaves probably approximates the natural field condition. Klement (1963) reported injection infiltration as the best method for rapid test of pathogenicity with phytopathogenic *Pseudomonas*. Horborg (1970) prepared an important apparatus for injecting solutions and suspension into thin leaves of plants to overcome the difficulties encountered by Kelment. Staar and dye (1965) considered the

syringe method as excellent but found it to be tedious and preferred pricking with multi needle for inoculation of phytopathogenic bacterial species into bean pods. Robson *et al.,(*1989) established hydathode inoculation method for the stimulation of natural black rot infection of cabbage *Xanthomonas campestris* pv. *campestris.* Chaudhury and Chakravarti (1989) found that among various artificial methods of inoculation *Xanthomonas campestris* pv. *ricini* , heavy infection developed when the leaves were inoculated after making injury with carborundum powder. Shah *et al*. (1995) observed that the bacterial blight of cowpea incited by *Xanthomonas campestris* pv. *viginicola* was more pronounced when the plants were spray inoculated twice at interval of 24 hr as compared to carborundum abrasion method. Though the incubation period was longer in spray inoculation, the method proved to be most convenient and suitable for field inoculation. Shekhawat and Chakravarti (1978) found that $6x10^3$ cells/ml for carborundum abrasion and $6 x10^5$ cells/ml for spray as minimal concentration for infection of *Xanthomonas vesicatoria* on chilli. Incubation period was reduced with the increase of number of cells in the inoculums. Singh (2001) found that a minimum inoculums concentration of 10^4 cfu/ml was necessary for infection and development of black rot disease of cabbage. However, higher concentration (10^8 cfu/ml) proved much better and induced high disease intensity.

MATERIALS AND METHODS

Different inoculation methods were applied for their pathogenecity test on the bacterium causing black rot of cabbage *in vivo* (pot experiment).

Bacteria Isolation

Xanthomonas campestris pv. *campestris* which was collected from the farmers field. The bacterial strain was isolated from infected leaves. Nutrient Agar a semisolid medium was used for bacterial isolation from infected cabbage leaves.

Infected leaves were cut into small pieces, which were then surface sterilized by immersing them 1% sodium hypochlorite solution for 1 min followed by two washings with distilled water. After that the leaf pieces were soaked in 50 ml sterile distilled water for 30 min at room temperature to allow bacteria to disperse into the surrounding liquid. After 30 min the water became cloudy which indicated the presence of a high number of bacteria. A loopful of the washings was streaked onto Nutrient Agar medium and incubated at 30°C for 48 h. After 48 h several yellowish watery colonies appeared on the plate. Further streaking on Nutrient Agar Petri plates allowed single colonies and pure cultures to be obtained.

Plant Germination and Growth for Pathogenicity Tests

The seedlings (var. *Golden Acre*) are raised in clean tin trays, using the mixture of compost and sandy loam soil in the seedbed. The trays were covered with mosquito net to protect the seedlings from bird and insect damage. The trays were watered as and when required. The one month old plants were transplanted in 8 inches diameter pot keeping one plant in each pot. Pots with plants were kept in a growth cabinet. Pathogenicity tests were conducted by inoculating one month old cabbage plants transplanted.

Inoculum Preparation

To maintain uniformity of inoculums, the bacteria growing on yeast extract glucose chalk agar slants, were gently

brought into suspension by adding 10 ml of sterile distilled water per culture tube and scrubbing the bacterial growth. The suspension so obtained was centrifuged at 5000 rpm for 10 min, supernatant discarded and the pellet was re-suspended and the process was repeated thrice by adding fresh 10 ml of sterile distilled water after each centrifugation. The finally washed suspension was used as inoculums and adjusted to a final concentration of 10^8cfu/ml.

Experimental Design

The experiment was done using five leaves for each plant with each bacterial inoculation method in a completely randomized design. Each leaf was considered as a replicate of each treatment.

Pathogenecity Test Using Different Inoculation Methods *Xanthomonas Campestris Pv. Campestris*

Six different methods are (1) Spraying the inoculums with hand atomizer (Klement, 1968), (2) Carborundum abrasion method (Leben et al., 1968) (3) Multineedle pricking method (Andrus, 1948; Starr and Dye, 1965) (4) Injection infiltration method (Klement, 1963) (5) Hydathodes inoculation method (Robeson et al., 1989) and (6) scissor clipping were studied to find out the efficiency of different methods in disease development. Inoculated plants were covered with polythene bags and kept under high humid condition for 48 h and then kept under natural condition. The plants were watered and regularly observed for disease development. The data so obtained are presented in

Spraying the Inoculums with Hand Atomizer (Klement, 1968)

The pathogen usually enters the tissue through stomata, lenticels, hydathodes this method of inoculation is the most natural. The plants were kept in humid condition prior to inoculation to allow the stomata to open and to create high intercellular humidity in the tissues around the natural openings. The bacterial suspension (1 x10^8 cfu/ml) was sprayed on cabbage plants with hand atomizer. The inoculated plants were kept under high humid condition for 48 h by covering them with polyethylene bags and then left as such under natural condition .

Carborundum Abrasion Method (Leben et al., 1968)

The plants were inoculated with help of cotton swab on both the surfaces of leaves. The cotton swab was soaked in inoculums containing carborundum power (300 mesh) for making gentle injury and application of inoculums simultaneously.

Spraying Inoculation after Multineedle Pricking (Andrus 1948; Starr and Dyes, 1965)

The leaves were injured with the help of multineedle prepared by fixing 8-10 fine pins with the help of a rubber band tightly. The injury to the leaves to the leaves was gentle so that it did not tear or perforate leaf surface. The bacterial suspension was then atomized on the leaf surface.

Injection Infiltration Method (Klement, 1963)

The method consists of injecting bacterial suspension into the intercellular spaces of leaves with a hypodermic needle. The hypodermic needle was inserted gently under the epidermis of the leaf. The opening face of the needle should be towards the leaf. Inoculations were made by injecting 0.1 ml of bacterial suspension in the leaf mesophyll so that tissue becomes water soaked.

Hydathode Inoculation Method (Robeson et al., 1989)

This technique was developed for the reliable inoculation of *Xanthomonas campestris* pv. *campestris* into the cabbage host, in a manner which simulates a natural process of penetration. In this method, the bacterial suspension was introduced into guttation droplets on the leaf margin and the bacteria were taken into the plant via the hydathode, thereby avoiding the mechanical injury to the plant and increasing the possibility of producing the disease symptoms.

Scissor Clipping

In this method the leaf margins were clipped in two to three places and the bacterial suspension was applied on the cut surfaces with the help of a cotton swab.

Suitable controls were maintained in each case using distilled water in place of inoculums suspension. The inoculated plants were kept under high humid condition for 48 h. The plants were then observed regularly for disease development. For all inoculations, 72 h old culture was used.

RESULTS

The data on lesion development was recorded 4 days after inoculation. *Xanthomonas campestris* pv. *campestris* isolate gave differential response with respect to method of inoculation. From the study it is revealed that the pathogen could successfully cause infection of the cabbage host plant by with varied incidence and intensity and some variation in symptom expression when inoculation was conducted by six inoculation techniques viz., Spraying the inoculums with hand atomizer, Carborundum abrasion method, Spraying inoculation after multineedle pricking, Injection infiltration method, Hydathode inoculation method and Scissor clipping methods. It is revealed from the data presented in table and figure that lesion progress was highest (89 mm) after 30 days of inoculation in case of Carborundum abrasion method followed by Hydathode inoculation method and Spraying inoculation after multineedle pricking methods giving average lesion progression of 72 and 61 mm, respectively after 30 days. The carborundum abrasion method was found most convenient and efficient method of inoculation for producing disease on potted plants.

DISCUSSIONS

In support of the present finding it was reported that in heavy infection developed when the leaves were inoculated after making injury with carborundum powder (Chaudhury and Chakravarti, 1989). Shah *et al*. (1995) observed that the bacterial blight of cowpea incited by *Xanthomonas campestris* pv. *viginicola* was more pronounced when the plants were spray inoculated twice at interval of 24 hr as compared to carborundum abrasion method. It was reported that vein inoculation method gave quicker symptom expression and highest lesion progression followed by Hydathode inoculation and Clip inoculation methods respectively. Klement (1963) reported injection infiltration as the best method for rapid test of pathogenicity with phytopathogenic *pseudomonas.*

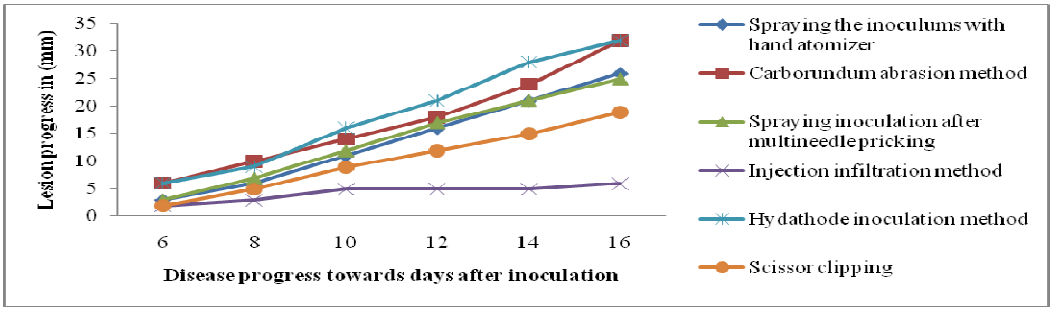

Figure 1: Lesion Progress (Mm) Using Different Inoculation Methods

Table 1: Lesion Progress by Using Different Inoculation Methods

Disease Progress Towards Days After Inoculation	Average Lesion Progress on (mm)					
	Spraying the Inoculums with Hand Atomizer	Carborundum Abrasion Method	Spraying Inoculation After Multineedle Pricking	Injection Infiltration Method	Hydathode Inoculation Method	Scissor Clipping
4	1	2	1	1	2	1
6	3	6	3	2	6	2
8	6	10	7	3	9	5
10	11	14	12	5	16	9
12	16	18	17	5	21	12
14	21	24	21	5	28	15
16	26	32	25	6	32	19
18	29	43	28	7	39	23
20	33	52	31	7	48	27
22	34	61	38	8	54	31
24	38	66	44	12	59	34
26	42	74	51	13	61	35
28	44	80	56	18	68	38
30	48	89	61	19	72	40

CONCLUSIONS

Amongst different inoculation methods tested, 'Carborundum abrasion method' gave quicker symptom expression and highest lesion progression in causing black rot of cabbage by X*anthomonas campestris* pv. *campestris*.

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