J Krishi Vigyan 2022, 10 (2) : 192-197 DOI : 10.5958/2349-4433.2022.00034.4

Host Range and Survival Studies of Bacterial Leaf Spot of Green gram Pathogen *Xanthomonas axonopodis* pv. *vignaeradiatae*

Jitendra Kumar[1]*, Renu Gupta[2] and Anila Doshi[3]

Rajasthan College of Agriculture, MPUAT, Udaipur 313001 (Rajasthan)

ABSTRACT

Bacterial leaf spot pathogen *Xanthomonas axonopodis* pv. *vignaeradiatae* produces symptoms on leaves, stems, pods and seeds. Small water-soaked spots are the initial symptoms observed on leaves and appear within 4 to 10d of infection. Bacterium was tested on 20 different plant species by inoculating one month old leaves using carborundum abrasion technique and the disease development was recorded. Out of the twenty plant species, the pathogen could produce visible symptoms on Black gram (*Vigna mungo*), cow pea (*Vigna sinensis*) and cluster bean (*Cyamopsis tetragonoloba*). This study indicated that sensitive plant species may be collateral hosts of this pathogen and it has narrow host range. In host age studies, the PDI decrease with increase in age of green gram plants after attaining the maximum severity at 30d stage. In plant debris, the survival period of pathogen was maximum (480d) in refrigerator and minimum (40d) in outdoor conditions. The bacterium survived longer (570d) in seeds, when soaked in bacterial suspension and stored in refrigerator.

Key Words: Bacterial blight, Green gram, Host range, Leaf spot, Suspension, Survival.

INTRODUCTION

Green gram (Vigna *radiata* L.) is one of the important pulse crops widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Vietnam, Indonesia, Malaysia and China (Kaur *et al*, 2014). Green gram plant suffers from two bacterial diseases namely, bacterial leaf spot and halo blight, the former is economically more important and widespread. Fang *et al* (1964) first reported bacterial leaf spot of green gram from China. Green gram crop has been often attacked by bacterial leaf spot and under severe infection infected leaves may dry prematurely, pods are also infected that leads reduced in yield. Infected green gram pods serve as source of infection in new area. It is having severe infection of bacterial leaf spot in Rajasthan ranging from 10 to 80 per cent in different areas (Gena *et al*, 2008). Bacterial leaf spot pathogen produces symptoms on leaves, stems, pods and seeds. Stem infection is less common and begins as a water-soaked spot, which becomes reddish-brown and usually without chlorosis. Severely infected seeds may be shriveled and show poor germination with weak plants (Chaudhary *et al,* 2019).

Bacterial blight of cowpea (*X. campestris* pv. *vignicola*) developed in the plant of all ages *i.e.,* 10 to 100 d but the disease was more on plants of age group between 20 to 60d (Shah *et al*, 1989). Thirty days old plants were, found to be most susceptible with the infection index of 60.8 percent. The disease was minimum on 100d old plants. Infection index decreased with increase in age of cowpea plants after attaining the maximum severity at 30 days. Thind (2012) described that age of the sesame leaves influences the severity of bacterial blight (*X. campestris* pv. *sesami*) disease. The disease index on older leaves was much less than

Corresponding Author's Email: jitpatho@gmail.com

[1]SMS - Plant Protection KVK, Fatehpur, Sikar-332301

[2]SMS – Plant Protection, KVK, Kotputli-303108

[3]Ex Dean, Rajasthan College of Agriculture, MPUAT, Udaipur 313001, Rajasthan,

Sr. No.	Host group	Plants used	Scientific name
1	**Pulses**	Urdbean	*Vigna mungo* L.
		Cowpea	*Vigna sinensis* Endl.
		Lentil	*Lens esculentus* Moench
		Cluster bean	*Cyamopsis tetragonoloba* (L.) Taub.
		Rajma	*Phaseolus vulgaris* L.
2	**Cereals**	Wheat	*Triticum aestivum* L.
		Barley	*Hordeum vulgare* L.
		Maize	*Zea mays* L.
		Sorghum	*Sorghum vulgare* Pers.
		Pearl millet	*Pennisetum typhoides* (Burm. f.) Stapf & C.E. Hubb.
3	**Oilseeds**	Sunflower	*Helianthus annuus* L.
		Groundnut	*Arachis hypogaea* L.
		Soybean	*Glycine max* Merr.
		Safflower	*Carthamus tinctorius* L.
		Mustard	*Brassica campestris* var. *sarson* Prain
		Castor	*Ricinus communis* L.
4	**Weeds**	Jungle rice	*Echinochloa colona* (L.) Link
		Day flower	*Commelina communis* L.
		Hazardana	*Phyllanthus niruri* L.
		Horse purslane	*Trianthema portulacastrum* L.

that on fully expanded young leaves. Multiplication of the pathogen in the artificially inoculated leaves reached maximum on 3rd day in both young and old leaves but the number of bacterial cells in young leaves was 10 times more than those in old leaves.

Seed borne inoculum forms an important source of primary inoculum and can initiate an epidemic on a large number of plants. The bacterial pathogen is seed borne and enters the host through stomata. Bacterium survived for one year in the seeds and inoculation of the bacterium only at inflorescences and at flat green pod stage led the bacterium to become seed-borne. Deformity of seeds like shriveling, discolouration and small size was only an indication but not a sure test of seed-borne infection. The detection of *X. axonopodis* pv. *vignaeradiatae* from green gram seeds and found that traditional growing on tests was more suitable in monsoon season when conditions were highly favourable for disease development while incubation growing on test proved more effective in summer crop season (Rathore, 2010).

Seed borne disease of green gram like bacterial leaf spot caused by *X. axonopodis* pv. *vignaeradiatae* has been reported from all regions of Rajasthan in mild to severe form. It causes considerable damage by reducing seed quality and yield. Information with respect to the mode of survival of the pathogen from one season to another is hardly available in the literature. Therefore, attempts were made post crop season to observe the role of infected seeds and crop debris in the perpetuation of the pathogen from season to season. Seed borne inoculum helps seed to spread further in new areas. The purpose of this work was to study the host range and survival of an isolate of *X. axonopodis* pv. *vignaeradiatae* and to assess its potential as a source of inoculum in the next growing season.

Table 1. Evaluation host range of *Xanthomonas axonopodis* pv. *Vignaeradiatae.*

Plants used	Scientific name	Reaction	Incubation Period (hrs)
Urd bean	*Vigna mungo* L.	+	56-58 h
Cowpea	*Vigna sinensis* Endl.	+	61-63 h
Cluster bean	*Cyamopsis tetragonoloba* (L.) Taub.	+	53-55 h

+ = Visible symptoms

Table 2: Effect of age of the plants on severity of bacterial leaf spot in green gram under conditions

Age of plants (Days)	Mean per cent disease index (PDI)		
	2013	2014	Pooled
10	32.20(34.563)*	31.40(34.071)	31.80(34.32)
20	39.50(39.165)	38.50(38.351)	39.00(38.76)
30	62.50(52.246)	59.70(50.604)	61.10(51.42)
40	47.10(43.336)	46.60(43.051)	46.85(43.19)
50	30.40(33.457)	28.20(32.075)	29.30(32.77)
60	25.60(30.389)	23.80(29.191)	24.70(29.79)
SEm±	0.5938	0.5162	0.3934
CD at 5%	1.7643	1.5336	1.1283
CV %	3.06	2.72	2.90

* Figures in the parenthesis are arc sin transformed values

Table 3: Survival of *Xanthomonas axonopodis* pv. *vignaeradiatae* in green gram plant debris stored under different conditions

Treatment	Survival period (days)
Refrigerator (5-10 ^{0}C)	480
Room (10-40 ^{0}C)	270
Cage house	110
Under a tree	90
Soil at 10 cm depth	120
Soil surface	60
Roof of laboratory	40

MATERIALS AND METHODS

Host range of the pathogen

Present investigation was conducted at research field of Rajasthan College of Agriculture, Udaipur by taking twenty different cultivated and wild plant species, which are being grown in *Kharif* and *Rabi* season. Host range of bacterial leaf spot pathogen *Xanthomonas axonopodis* pv. *vignaeradiatae* was tested on 20 different plant species by inoculating one month old leaves using carborundum abrasion technique and the disease development was recorded, if any. Suitable controls were maintained in each case. Following plants were used in host range studies,

Table 4: Survival of *Xanthomonas axonopodis* pv. *vignaeradiatae* in green gram seeds stored inside a refrigerator (5-10 ^{0}C) and at room (10-40 ^{0}C) temperature

Seed	Survival period (days)	
	Refrigerator (5-10 ^{0}C)	Room (10-40 ^{0}C)
Udaipur market	340	210
Farmers Field (diseased)	360	240
Spray Inoculated field	390	280
Soaked in bacterial suspension for 6 hrs	570	350

To study the host-range of bacterial leaf spot of green gram pathogen, some of the cultivated plants and wild plants were raised in 25 cm earthen pots in cage house. One month old plants of 20 plants species, belonging to different families, were inoculated by bacterial suspension ($2.5x10^8$cfu/ml) with carborundum abrasion technique. The inoculated plants along with controlled plants were kept under high humidity (85-90%) conditions for 48 hr and then these were placed in cage house. In control, sterilized distilled water was sprayed. The pathogenicity was proved by following Koch's postulates in those plants which showed infection. The observations were recorded using standard rating scale for disease severity and depicted as present (+) and absent (-).

The effect of host age on disease development

The effect of host age on bacterial leaf spot development was studied by spray inoculating green gram plants of different age groups varying from 10, 20, 30, 40, 50 and 60 days with bacterium. The plants were raised in 25 cm. earthen pots by sowing green gram at an interval of 10 days. In each pot ten plants were grown and four pots were used for each age group study. All the plants were spray inoculated with a bacterial suspension ($2.5x10^8$cfu/ml) thrice at 24 hrs interval and final observation for disease intensity was recorded 15 days after inoculation for each age group plants.

Survival of bacterium

Survival of bacterium in infected host tissues and seed was studied under varying indoor and outdoor conditions to understand the method of its perpetuation and recurrence of disease in this area. For indoor conditions, the diseased plants parts were stored in laboratory (10-40 ^{0}C) and refrigerator (5-10 ^{0}C). For outdoor conditions, diseased samples were kept on soil surface in the field, on roof of laboratory, in cage house, under tree shade and buried 10 cm deep in the field soil. Isolations from each lot were made at 10 days interval on YGCA media and pathogenicity of bacterium, so developed, was tested.

Studies were made to understand the role of seeds in survival of the green gram leaf spot bacterium. The period of bacterial survival in green gram seeds was determined by storing four different seed samples *viz.,* seeds collected from Udaipur local market, naturally infected farmers fields, spray inoculated fields and healthy seeds soaked in bacterial suspension ($2.5x10^8$cfu/ml) for 6 hrs. A sample of 1 kg seeds was used for each treatment. All four type of seeds lots were kept in plastic containers at room temperature (10-40^{0}C) and refrigerator (5-10^{0}C). Ten seeds from each lot were picked up at 10 day interval, crushed in 10 ml sterile water, stirred thoroughly and supernatant was streaked on YGCA media to observe bacterial growth, if any.

RESULTS AND DISCUSSION

Host range of the pathogen

The result of host range studies revealed that the pathogen causing bacterial leaf spot of green gram produced visible symptoms on urdbean

(*Vigna mungo*), cowpea (*Vigna sinensis*) and cluster bean (*Cyamopsis tetragonoloba*) however, the symptoms did not appear on rest of the plants. The symptom expression and incubation period was between 56-58 hr in Urdbean (*Vigna mungo*), 61-63 hr in cowpea (*Vigna sinensis*) and 53-55 hr in cluster bean (*Cyamopsis tetragonoloba*). The pathogen was re-isolated from tested plant leaves and the morphological characters of the re-isolated pathogen were compared with the original culture, these were found to be similar in all respects. Hence, results indicated that green gram pathogen *Xanthomonas axonopodis* pv. *vignaeradiatae* has narrow host range. Similar studies were carried out by Thind (2012) that in addition to green gram the bacterium (*X. axonopodis* pv. *vignaeradiatae***)** was moderately virulent on bean, lentil and Indian bean whereas, this bacterium was found to be weakly virulent on lima bean under artificial inoculations conditions. Further, their role in the perpetuation of the disease has not been demonstrated, so far (Table 1 & Fig. 1).

Effect of age of the host on disease development

Age of the host plant is an important factor for infection and development of any disease. In the present investigation, thirty days old plants were found to be most susceptible with the infection index of 61.10. The disease was minimum on 60 d old green gram plants. Percent disease index decreased with increase in age of cowpea plants after attaining the maximum severity at 30 d stage (Table 2). Further, the plants of 30 to 40 d age were found to be most susceptible. However, at this age all the leaves present on the plant developed infection. The fact that older leaves were found to be less susceptible to bacterial leaf spot infection, indicates possibility of developing resistant in leaves with age owing to altering host physiology. The present findings were strengthened by the study of Shah *et al* (1989) who while working with bacterial blight of cowpea (*X. campestris* pv. *vignicola*) observed that disease was more severe on plants of age group between 20 to 60 d.

Survival of bacterium

In the present investigation, the possible methods of survival of green gram leaf spot bacterium were explored to understand the manner of its recurrence in this area. The bacterium *X. axonopodis*pv. *vignaeradiatae* was found to survive in plant debris for 480d when stored in refrigerator and for 270d at room temperature. Direct heat of sun had adversely affected the survival of green gram bacterium present in host tissues and could survive for only 40 d when the infected tissues were kept in open on a roof during summer months (Table 3).

Plant debris and seed borne inoculum forms an important source of primary inoculum and can initiate an epidemic on a large number of green gram plants (Rathore, 2010). Bacterial blight of cow pea pathogen (*X. axonopodis* pv. *vignicola*) did not survive for more than three weeks in diseased plant debris under field condition.

Soni (1982) established the seed-borne nature of the bacterium and showed that seed is an important source of survival for the bacterium. He further reported that the bacterium survived for one year in the seeds and inoculation of the bacterium at inflorescences and at flat green pod stage led the bacterium to become seed-borne. The infected seed could we detected by visual examination by their small size, darker colour and small patches on the surface. In the present studies also, the bacterium was found to survive in seed samples collected from farmer's field, for 360d at 5-10 ^{0}C and 240d at room temperature. By above observations it can be inferred with certainty that seed is the primary source of inoculum for green gram bacterial leaf spot in this area (Table 4).

CONCLUSION

The host range of *Xanthomonas axonopodis* pv. *vignaeradiatae* revealed that bacterium can produce visible symptoms only on black gram, cow pea and cluster bean. Therefore, leaf spot of green gram pathogen has narrow host range. The severity of the disease was more on plants of age group between 20

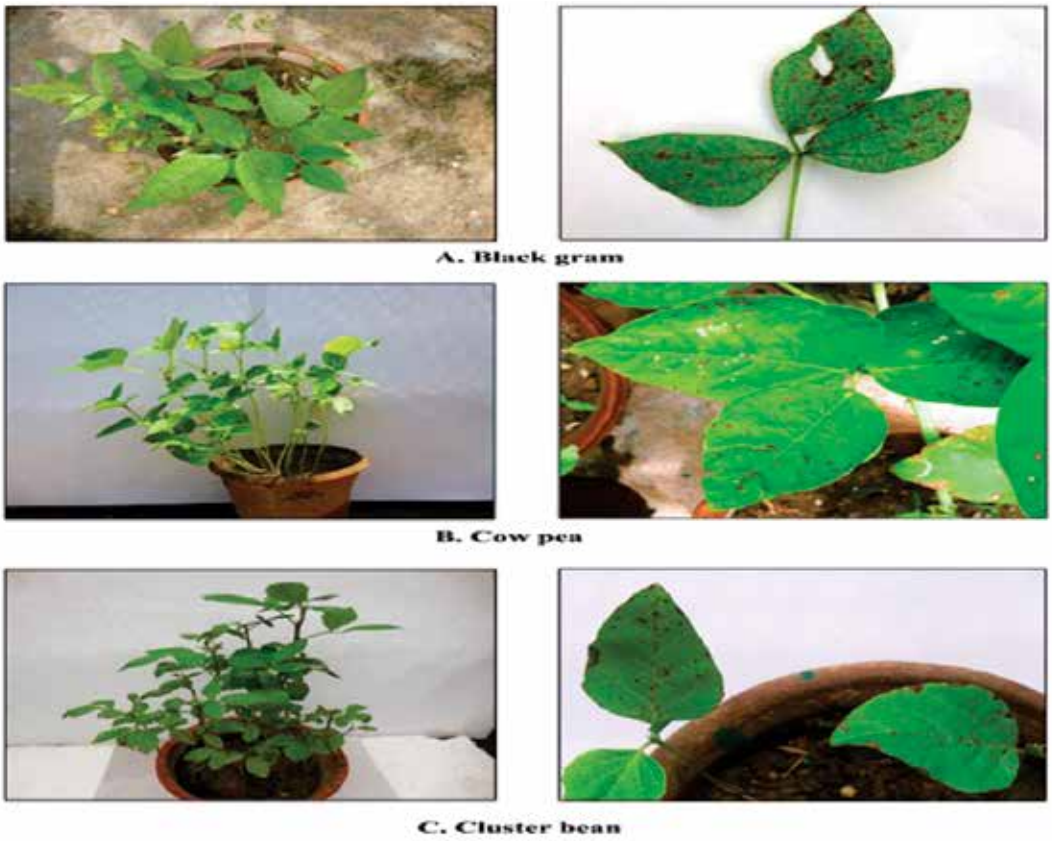

Figure 1: Evaluation of host range of *X. axonopodis* pv.*vignaeradiatae* under pot house conditions

to 40 d. However, thirty days old plants were found to be more susceptible and disease was minimum on 60 d old plants. In plant debris, during laboratory conditions the maximum survival of pathogen was in refrigerator and survival period was much shorter in outdoor conditions. This suggests that direct heat of sun has a detrimental effect on the bacterium present in diseased host tissues. The bacterium survived longer in seeds, when it was stored at low temperature. The survival period of bacterium at higher temperature was much shorter.

REFERENCES

Chaudhary M, Singh K, Khokhar MK, Rawal P and Shah R. (2019). Management of bacterial leaf spot of green gram caused by *Xanthomonas axonopodis* pv. *Vignaeradiatae. J Mycol and Plant Pathol* **149 (1),** 67-72.

Fang C T, Chen HY and Chu CL (1964). A comparative study of the species of the genus from leguminous plants. *Acta Phytopath* Sinicia **7**, 31-23.

Gena M, Shah R and Mali BL (2008). Host range and efficacy of agrochemicals, botanicals and bacilli against cowpea blight bacterium *Xanthomonas axonopodis* pv. *vignicola*. *J Plant Dis Sci* **3,** 144-150.

Kaur P, Kaur A, Kaur B and Singh K (2014). Performance of front line demonstrations on summer moong in Jalandhar district. *J Krishi Vigyan* **3 (1)**, 58-61.

Rathore BS (2010). Efficacy of streptocycline and plant extracts against bacterial leaf spot green gram. *J Mycol and Plant Pathol* **36,** 153-156.

Shah R, Bhatnagar MK and Mali BL (1989). Epidemiology of bacterial blight of cowpea. *7th International Conference on Plant Pathology and Bacteriology*, Budapest, Hungry, pp. 323-328.

Soni PS (1982). Detection of phytopathogenic bacteria from cowpea and mung seeds. Ph. D. Thesis. Punjab Agricultural University, Ludhina, pp. 180.

Thind BS (2012). *Phytopathogenic prokaryotes and plant diseases.* Scientific Publishers, Jodhpur, pp. 401-470.

Received on 27/4/2021 *Accepted on 10/1/2022*