Research Article

Management of Bacterial Leaf Spot of Greengram Caused by *Xanthomonas axonopodis* pv. *vignaeradiatae*

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Abstract

Green gram is an important pulse crop, which is grown all over world for its nutritional value and improvement of soil fertility. Bacterial leaf spot disease devastated green gram field in Rajasthan, caused by *Xanthomonas axonopodis* pv. v*ignaeradiatae,* with ranging severity from 10-80 per cent. Disease was observed initially on leaf, advanced to all parts of plants and it is transmitted to seed. Different artificial inoculation methods *viz*, single spray, double sprays at 24 h of interval, carborundum abrasion and single spray after multineedle pricking were standardized in which two sprays at 24 h interval which cause 68.80 per cent symptom of disease in plants was found most convenient and suitable for expressing symptom under glasshouse conditions. Efficacy of different fungicides and antibiotics were evaluated under in *vitro* and pot experiments. Among different treatments maximum per cent disease control was recorded in Labistryn 89.72 per cent followed by Streptocycline (85.12%) and Bacterinol.

Key words: Fungicides and antibiotics, green gram, *Xanthomonas axonopodis* pv. *vignaeradiatae*

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Green gram is most important source of dietary protein, and has unique property of maintaining and restoring soil fertility through nitrogen fixation as well as conserving and improving physical properties of soil by virtue of their deep root system and leaf fall. Green gram is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Vietnam, Indonesia, Malaysia and China. In India, it is grown over on an area of 39.4 lac hectares with a production of 21.30 lac tones with average productivity 546 kg ha^{-1} in 2016-2017. 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. In addition to India and China, it also occurs in Pakistan (Iqbal et al 2003).

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 during kharif 2012 and 2013. 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. Longitudinal lesions, slits or cankers extending from soil level to the growing tip on the stems of seedlings growing from severely infected seeds.Mungbean crop has been often attacked by bacterial leaf spot and under severe infection, infected leaves may dry prematurely, pods are also infected and plants may give reduced yield. Infected mungbean pods serve as source of infection in new area.

Rathore (2010) described that seed borne inoculum forms an important source of primary inoculum and can initiate an epidemic on a large

number of plants. Seed borne inoculum also helps seed to spread further in new areas. It was difficult to manage using only cultural practices therefore proposed study was undertaken to find out effective management of leaf spot disease of green gram. Bactericides can control other bacterial diseases of pulses. For bacterial leaf spot of green gram, bactericides have been evaluated as disinfectants for seed and applications for disease control in field condition.

Materials and Methods

Green gram plants of different cultivar were collected from fields located in Nagour district of Rajasthan during Kharif, 2012. For isolation of the causal bacterium, infected portions of plants were cut, surface sterilized for 2-3 min with 2 per cent sodium hypochlorite solution and washed thoroughly thrice with sterile distilled water. The bits were left for one min to allow bacterial ooze to come out in water. The inoculated plates were incubated at 28±2 C on yeast extract glucose chalk agar medium (Shah et al (1989). After 48 to 72 h obtained bright yellow coloured bacterial colonies. Typical colony of *X. axonopodis* pv. *vignaeradiatae* isolates identified based on colony character , morphology and molecular basis. (Vauterin et al 1995) DNA from one of the representative isolate of *X. axonopodis* pv. *vignaeradiatae* was isolated by adopting the CTAB method. For 16S rDNA amplification, prokaryotic universal primers 27F: 5' AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5' GGTTACCTTGTTACGACTT- 3' were used. PCR amplification was done as per standard protocol. Sequence was compared with GenBank sequences by BLAST analysis. Nucleotide sequence similarities were determined using the NCBI databases and the bacterial identity was established by closest match (Altschul et al 1997).

Pathogenicity tests were conducted by inoculating one month old green gram plants raised in 25 cm earthen pots by two methods (i) spraying the bacterial suspension and (ii) applying the bacterial suspension on leaves using 300 mesh carborundum powder (Gena et al 2008).

Production of disease by artificial inoculation is an essential requirement to understand various aspects of leaf spot disease. Green gram plants were inoculated by four different methods *viz.*, single spray inoculation of bacterial suspension once, double sprays inoculation of bacterial suspension at 24 h interval, carborundum abrasion and single spray inoculation after multineedle pricking to find out suitable method of disease development for mass inoculation in potted plants. In each inoculation method 20 greengram plant was inoculated. All inoculations were made on one month old potted green gram plants during kharif season in cage house when general humidity conditions were very high.

The following disease rating key was devised based on bacterial leaf spot disease development in which infected plants were categorized in six arbitrary classes (Scale 0-5) :

Disease Rating	Severity of disease (%)	Description
0	0.0	No infection
1	0.1-10	Minute water soaked spots scattered over the leaves
2	11-20	Little bigger spots covering about 20-25 per cent leaf area.
3	21-40	Bigger leaf spot covering about 25-50 per cent leaf area, few small spots on petiole and stem initiated.
4	41-70	About 50- 75 per cent leaf area covered by heavy necrotic spotting, distinct enlarged elliptical lesion on petiole and stem.
5	Above 71	Above 75 per cent leaf area covered with necrotic leaf spot, cracking of stem, leaf spot or infection on pods

$$\text{Per cent infection index} = \frac{\text{Sum of individual ratings}}{\text{Number of plants leaves assessed} \times \text{Maximum disease rating}} \times 100$$

Management of bacterial leaf spot of green gram by *in vitro* evaluation of fungicides and antibiotics each chemical at five concentrations was evaluated for their efficacy against the growth of *X. axonopodis* pv. *vignaeradiatae* by paper disk method. Solution of different fungicides and antibiotics solution were prepared at desired concentrations. The filter paper discs (Whatman No. 42) measuring 10 mm in diameter were soaked in the respective chemical solution for 10 min and transferred onto the surface of the seeded medium in petriplates (3 disc/ plate). The inoculated plates were kept in the refrigerator at 5C for 4 h to allow the diffuse of chemicals into the medium. Then, plates were incubated at 28±2C for 72 h and observed for the production of inhibition zone around the filter paper discs. This experiment was repeated three times. Observations were recorded for the production of inhibition zone representing the efficacy of plant extracts in inhibiting the growth of pathogen (Gena et al 2008).

Management of bacterial leaf spot of mungbean was carried out through threetype of experiments *i.e.* seed treatment, foliar sprays and combined seed treatment and foliar sprays were conducted during kharif season 2012 and 2013 in the Cage house, Department of Plant Pathology, RCA, Udaipur.For seed treatment experiment inoculated seed (1 h in bacterial suspension) were treated with different fungicides and antibiotics for 1 h at desired concentration and seed soaked in water served as control. Germination per cent was recorded after 10 days of sowing and per cent disease intensity at flowering stage of mungbean plants.

Foliar sprays experiments, efficacy of foliar sprays were determined by inoculating one month old crop plants twice at 24 h interval. After inoculations, plants were given overall two foliar sprays at an interval of seven days with desired component at the time of first appearance of symptoms (Bull et al 2005).

Per cent disease control was calculated by following formula:

$$\text{Per cent disease control} = \frac{\text{PDI in Control} - \text{PDI in Treatment}}{\text{PDI in Control}} \times 100$$

Mungbean fresh plant weight was also recorded for each treatment at flowering stage and per cent increase in fresh plant weight was calculated as under:

$$\text{Per cent increase in fresh plant} = \frac{\text{Fresh plant weight in treatment} - \text{Fresh plant weight in control}}{\text{Fresh plant weight in control}} \times 100$$

The result of various experiment were statistically analyzed by using Completely Randomized Blocked Design (CRD) designs.

Results and Discussion

Green gram leaf spot symptoms were brown, circular to irregular and raised. Generally the spots were dry and necrotic from the beginning of their formation but sometimes they are water soaked with translucent border. These were similar to observed by Thind (2012). Isolations were made by streaking plate method on nutrient agar medium. Small yellow circular, smooth and shining colonies were obtained. The bacterium is rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative, capsulated, non-spore forming, having single polar flagellum and measured 0.4 – 0.25 um to 1.25 – 3.25 µm in size. The cells readily stained with common stain such as crystal violet, gentian violet and carbol fuchsin. Representative isolates obtained from diseased area of Merta Dist Nagaur used molecular identification. Sequence comparison of the 16S rDNA gene (1400bp) with GenBank entries further confirmed the identity as the similarity percentage was above 98.0 per cent to that of *Xanthomonas axonopodis* entries in the database. The 16S rDNA gene sequence of the isolate was assigned GenBank accession No. MH560278. Based upon morphological and moleculerer basis bacteria was identified as *Xanthomonas axonopodis* pv. *vignaeradiatae*.

Green gram plants were inoculated by four different methods indicated that green gram leaf spot pathogen could infect plants irrespective of the method used. The early expression of disease (5-6 days) was noted in both double sprays and carborundum abrasion followed by single spray and multineedle pricking. It was noted that maximum

Table 1. Methods of inoculation for the development of bacterial leaf spot in potted Green gram plants

Inoculation methods	Incubation period (Days)	Average per cent infection index
Single spray	7-8	41.27
Double sprays at 24 h interval	5-6	68.80
Carborundum abrasion	5-6	59.02
Spray inoculation after multineedle pricking	6-7	35.16

Table 2. *In vitro* efficacy of fungicides against bacterial pathogen after 72 h of incubation at 28±2 C plants

S.No.	Fungicide	Inhibition zone (mm)*				
		500	1000	1500	2000	2500
1	Blitox	3.40	4.50	7.90	10.40	13.10
2.	Kocide	2.40	3.80	5.20	6.60	8.10
3.	Carbendazim	1.10	1.40	1.90	3.20	3.90
4.	Control	0				

	SEm±	CD at 5%	CD at 1%
Fungicide	0.0762	0.2154	0.2866
Concentration	0.09832	0.2781	0.37
Fungicide x Concentration	0.1703	0.4817	0.6409
CV %		4.2855	

* Average of five replication

disease (68.80 per cent) developed in double sprays at 24 h interval followed by carborundum abrasion method (59.02 per cent), single spray inoculation (41.27 per cent) and single spray with multineedle pricking (35.16 per cent). Double sprays at 24 h interval were found most convenient and suitable method of inoculation for developing disease. The results of present finding were similar to the Shah et al (1995) and Gena (2006) in case of bacterial blight of cowpea.

For management of green gram bacterial leaf spot three fungicides *viz*. Blitox, Kocide and Carbendazim were tested for their efficacy against the growth of green gram leaf spot bacterium by paper disc method at 500,1000,1500,2000 and 2500 ppm concentrations under *in vitro* condition, All three able to reduce the growth of green gram leaf spot bacterium (Table 2). However, Blitox was found superior, followed by Kocide and Carbendazim in all five concentrations tested. Five antibiotics were tested for their efficacy against the growth of green gram leaf spot bacterium by paper disc method at five concentrations. The zone of inhibition was measured after 72 h incubation and average data are presented in Table 3. All five antibiotics used were found effective against the growth of test bacterium. However, Labistryn inhibited maximum growth of the test bacterium that followed by Streptocycline, Bacterinol, Plantomycin and Erocin, respectively. Rathore (2010) also found Streptocycline as best against this disease. Fungicides, Blitox remained better than Kocide and Carbendazim in current study and similar reports have been reported by Jindal and Thind, 1994.

Table 3. *In vitro* efficacy of antibiotics against bacterial pathogen after 72 h of incubation at 28±2 C plants

S.No.	Bactericide	Inhibition zone (mm)*				
		500	1000	1500	2000	2500
1	Streptocycline	7.50	11.80	16.50	19.20	24.30
2.	Labistryn	8.80	12.90	17.10	20.50	26.10
3.	Plantomycin	6.10	10.40	15.10	17.80	22.90
4.	Bacterinol	6.90	11.20	15.90	18.60	23.70
5.	Erocin	4.60	8.90	13.60	16.30	21.30
6.	Control	0				

	SEm±	CD at 5%	CD at 1%
Antibiotic	0.0867	0.2433	0.3221
Concentration	0.08672	0.2433	0.3221
Antibiotic x Concentration	0.1939	0.5441	0.7202
CV %		2.8563	

* Average of five replication

Table 4. Evaluation of fungicides, antibiotics, botanicals and bioagents as seed treatment and foliar sprays against bacterial leaf spot of mungbean in potted plants

Treatment	Per cent disease index (PDI)	Per cent disease control (PDC)	Fresh plant weight (g/plant)	Per cent increase in fresh plant weight
Blitox	37.48	47.78	96.82	34.44
Kocide	43.88	38.87	94.32	30.97
Carbendazim	46.58	35.11	91.22	26.66
Streptocycline	10.88	85.12	121.62	68.88
Labistryn	7.58	89.72	126.12	75.13
Plantomycin	12.88	82.33	108.62	50.83
Bacterinol	11.68	84.01	109.92	52.63
Control	71.78		72.02	–
SEm±	0.9929		1.0988	
CD at 5%	2.8178		3.1182	
CD at 1%	3.7560		4.1564	
CV%	7.11		5.45	

* Average of 2 years data

In pot trials, 2 years data was pooled in which antibiotics remained superior over fungicides in reducing the leaf spot disease in plants. Labistryn with percent disease control of 89.72 per cent was found best followed by Streptocycline (85.12%) and Bacterinol (84.01) against the leaf spot disease. Minimum disease and highest fresh plant weight of mungbean were also obtained by Labistryn which

was 75.13 per cent increase in fresh plant weight over control pot followed by Streptocycline and Bacterinol when used as two foliar sprays and seed treatment + two foliar sprays. This result was similar to Gena (2008) in which they found Labistryn as best followed by Streptocycline against bacterial blight of cowpea. Rathore (2010) also found Streptocycline as best against this disease.

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

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