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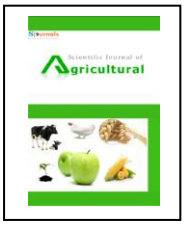

Original article

Survey, surveillance and cultural characteristics of bacterial blight of soybean

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ABSTRACT

A survey was undertaken in eight districts *viz.,* Parbhani, Nanded, Hingoli, Beed, Osmanabad, Jalna, Latur and Aurangabad districts of Marathwada region during June-August 2009-10. In all 69 soybean fields were surveyed by road for recording disease severity and incidence of blight of soybean. The pod highest disease severity noticed on the soybean field of Parbhani district followed by Hingoli, Nanded, Latur and Beed . The JS-335 showed maximum pod blight severity in all surveyed districts. The average per cent disease incidence (14.5%) in Marathwada region was recorded. Highest disease incidence noticed in Parbhani district (23 per cent) followed by Hingoli (20 %), Nanded (17 %), Latur (15 %) and Beed (13 %). The lowest disease incidence noticed in Jalna district (7 %). The pathogen was isolated, purified and its pathogenicity was proven in pot culture. Further, on the basis of morphological, cultural characteristics of the pathogen and symptomatology, the fungal pathogen was identified as *Pseudomonas syringae*.

1. Introduction

Soybean belongs to the family *Leguminoseae* and sub-family *Papilionoidae.* The crop is presently grown in most of the part of the world and is a primary source of vegetable oil and protein. It contains 44.65 per cent

protein, 8.77 per cent fats, 27.12 per cent nitrogen, 5.89 per cent ash, 5.96 per cent fibre. In India area, production and productivity of soybean during 2007-2008 were 79.720 lakh ha., 64.28 lakh metric tonnes and 802 kg/ha., respectively (Anonymous, 2007).

Soybean crop is know to be affected by more than 100 plant pathogens out of 35 are of economic importance. Among the major bacterial, bacterial blight caused by *Pseudomonas syringae* has been reported yield loss potential for this disease to range from 4 per cent to as high as 40 per cent under extreme condition (Meshram and Sheo-raj, 1988 and 1992; Lim, 1992 and Mishra and Krishana *et al*., 2001). Considering the variable types of wilt reactions of the released variety in the farmer's field and sick plot at different locations and yield losses caused, this investigations were undertaken to find out the major causal organism involved in chickpea wilt complex in Marathwada region of the Maharashtra state. Survey and surveillance of chickpea wilt complex incidence on farmer's field, collection, isolation, purification and pathogenicity of wilt pathogen was done.

2. Materials and methods

2.1. Survey and surveillance

A roving disease survey of the incidence and severity of bacterial blight disease of soybean during July to August 2009 was undertaken at Parbhani, Nanded, Hingoli, Beed, Osmanabad, Jalna, Latur and Aurangabad districts of Marathwada region. Total sixty nine soybean fields were inspected. Out of which 11, 5, 7, 11, 6, 9 ,10 and 10 were from, Parbhani, Jalna, Aurangabad, Hingoli, Osmanabad, Beed, Nanded and Latur districts respectively.

In Latur district Latur and Nilanga in Aurangabad District Aurangabad and Vaijapur, in Parbhani district Parbhani, Selu, Purna and Pathri, in Nanded district Nanded and Bhokar, in Hingoli district Hingoli and Basmat, in Beed district Beed, Kaij and Ambejogai, in osmanabad district osmanabed and Tuljapur and In Jalna district jalna and Badnapur tahsils were inspected for recording bacterial blight disease incidence and its severity.

Incidence of bacterial blight disease was exmined as severe, moderate, trace and free on the basis of percentage of severity of disease. Similarly, the per cent disease incidence of bacterial blight was calculated by using formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plant infected}}{\text{Total number of plant examined}} \times 100$$

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of all disease rating}}{\text{Number of ratings x Maximum disease grade}} \times 100$$

2.2. Isolation

Soybean plants naturally infected and blight with typical symptoms were collected from farmers' field and brought to the laboratory. All samples collected from different locations were subjected to isolation on nutrient agar (NA) in the laboratory.

2.3. Pathogenicity

Five seeds of the susceptible variety JS-335 were sown in each pot filled with soil and FYM in 2:1 proportion and immediately watered with sterilized distilled water. Two weeks after germination, only four seedlings were maintained in each pot. When the plants were 4 weeks old, a bacterial suspension (10^8 cfu/ml) was prepare as an inoculums for pathogenicity test. The underside of the leaf surface was sprayed with water and dusted with carborendum powder. Further, these leaves were smeared with bacterial suspension by means of sterile cotton swab. These pots were placed in a polyhouse, where high humidity and optimum temperature (24 ± 2 ^{o}C) were maintained for further development of bacterial blight disease symptom. After eight days incubation period, typical symptom on foliage of artificially diseased (bacterial blight) soybean plants were observed. The organism was reisolated on nutrient agar medium.

2.4. Cultural studies

Growth characters of the isolated *Pseudomonas syringae* was studied by growing it on different nutrient agar culture media. The media used were Nutrient agar, Potato dextrose agar, Yeast extract mannitol agar and Glucose chalk agar medium. These agar media were prepared by following standard laboratory procedure, sterilized by autoclaving, poured into the sterile Petri plates, (ten plates of each medium) and allowed to cool down and solidify. Then, the plates were inoculated by bacterium and incubated at room temperature.

Table 1
Survey of bacterial blight severity of soybean in different districts of Marathwada region.

Sr. No.	District	No. of field surveyed	Variety	Disease severity				
				Severe	Moderate	Trace	Free	Total
1	Parbhani	11	JS-335	2	2	1	-	5
			MAUS-81	1	1	1	-	3
			MAUS-71	-	1	1	1	3
			Total	3	4	2	1	11
2	Nanded	10	JS-335	1	2	-	1	4
			MAUS-81	1	1	1	-	3
			MAUS-71	1	1	1	-	3
			Total	3	4	2	1	10
3	Hingoli	11	JS-335	2	2	1	1	6
			MAUS-81	1	1	1	-	3
			MAUS-71	1	1	-	-	2
			Total	4	4	2	1	11
4	Beed	9	JS-335	1	1	2	-	4
			MAUS-81	-	1	1	1	3
			MAUS-71	1	1	-	-	2
			Total	2	3	3	1	9
5	Osmanabad	6	JS-335	1	-	1	1	3
			MAUS-81	1	1	1	-	2
			MAUS-71	-	1	-	-	1
			Total	2	2	2	-	6
6	Jalna	5	JS-335	1	1	1	-	2
			MAUS-81	-	1	1	-	2
			MAUS-71	-	1	-	-	1
			Total	1	3	2	-	5
7	Latur	10	JS-335	2	1	1	1	5
			MAUS-81	1	2	-	-	3
			MAUS-71	-	1	1	-	2
			Total	3	3	1	-	10
8	Aurangabad	7	JS-335	1	1	1	-	3
			MAUS-81	1	2	-	-	3
			MAUS-71	-	1	-	-	1
			Total	2	3	1	-	7

Severe : > 50%
Moderate : > 10% to < 50%
Trace : < 10%

3. Results and discussion

Results (Table 1 and Fig 1) revealed that the severity of disease was maximum in Parbhani district followed by Hingoli, Nanded, Latur and Beed. The disease severity observed minimum in Jalna district. The result Table 1

clearly depicts that the variety JS-335 is highly susceptible to disease in all districts followed by MAUS-81 and MAUS-71.

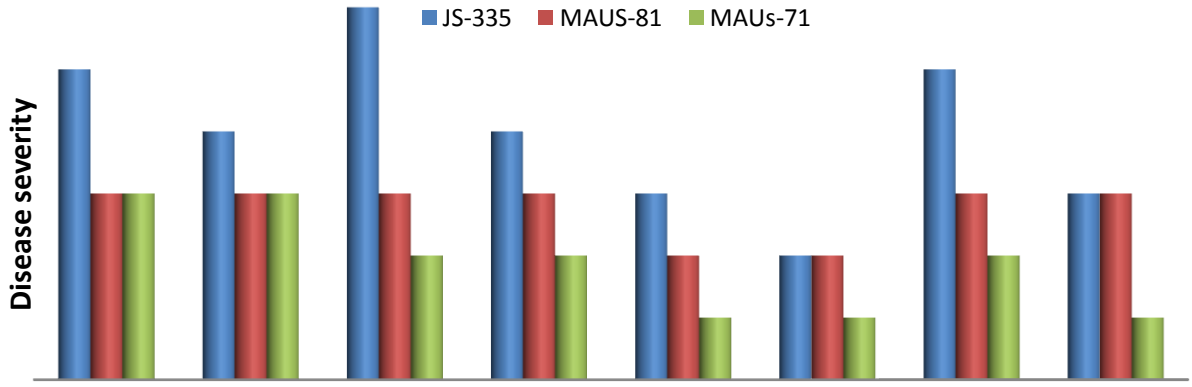

Fig. 1. Survey of bacterial blight severity of soybean in different districts of Marathwada region.

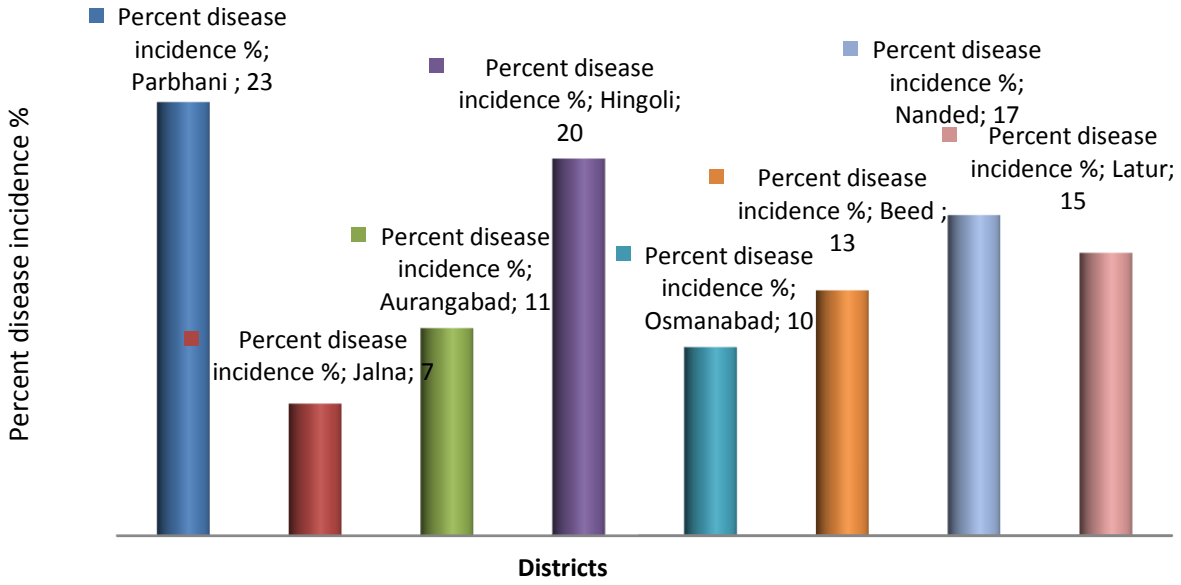

Fig. 2. Per cent disease incidence of bacterial blight of soybean in different districts of Marathwada region.

Results (Table 2 and Fig 2) clearly revealed that the maximum per cent disease incidence in Parbhani district (23 per cent) followed by Hingoli, (20 %), Nanded (17 %), Latur (15 %), Beed (13 %), Aurangabad (11 %) and Osmanabad (10 %). The minimum per cent disease incidence of bacterial blight of soybean was found in Jalna district (7 %). This result was similar to Song shun *et al.,*(2002).

Due to this disease, the soybean yield was reduced substantially. The average per cent disease incidence in Marathwada region was about 14.5 per cent. In all sixty nine fields were surveyed and average disease incidence (PI) to the tune of 14.5 per cent has been observed. Highest disease incidence noticed in Parbhani followed by Hingoli, Nanded and Latur district. Highest disease severity noticed in Parbhani district followed by Hingoli, Nanded, Latur and Beed.

3.1. Pathogenicity

Five seeds of the susceptible variety JS-335 were sown in each pot filled with soil and FYM in 2:1 proportion and immediately watered with sterilized distilled water. Two weeks after germination, only four seedlings were maintained in each pot. When the plants were 4-6 weeks old, a bacterial suspension (10^8 cfu/ml) was prepare as an inoculums for pathogenicity test. The underside of the leaf surface was sprayed with water and dusted with carborendum powder (Plate 3).

These pots were placed in a glasshouse, where high humidity and optimum temp (24 $\pm$2^{o}C) were maintained for further development of bacterial blight disease symptom. After two weeks incubation period, typical symptoms on foliage of artificially diseased (bacterial blight) soybean plants were observed. The organism was reisolated on nutrient agar medium and pathogen is confirmed as P. syringae.

Table 2

Per cent Disease incidence of bacterial blight of soybean in different districts of Marathwada region.

Sr.No.	District	Total no. of field surveyed	Total no. of plants examined	Infected plants with bacterial blight	Per cent disease incidence (%)
1	Parbhani	11	300	67	23
2	Nanded	5	200	14	17
3	Hingoli	7	250	27	20
4	Beed	11	300	30	13
5	Osmanabad	6	200	20	10
6	Jalna	9	250	32	7
7	Latur	10	250	47	15
8	Aurangabad	10	250	37	11
Average					14.5

3.2. Identification of fungal pathogen

The pathogenic culture isolated from the diseased plants was identified on the basis of the morphological characters as *P. syringae* and was confirmed from NIKU, BIO-Research Lab and PUNE-411002.

The results in respect of the growth characters of *P. syringae* on various culture media are presented in Table 3, 4 and 5. From the results, it was revealed that the moderate growth was obtained on medium Yeast extract mannitol agar broth and Glucose chalk Agar Broth of susceptible variety JS-335. All the three media proved to be significantly superior in favoring the growth of *P. syringae* than the rest of the media tested. The poor growth was observed on Nutrient broth.

Table 3

Growth and cultural characters of *Pseudomonas syringae* pv. *glycinea* on different agar media in petri plate.

Sr.No.	Medium	Growth characters after 48hrs of incubation
1.	Nutrient agar	Growth abundant, filiform, slightly raised, glistering, butyrous, pale yellow fluorescent secondary colonies begin to develop along the margin.
2	Potato dextrose agar	Growth fairly good, filirom, slightly raised, glistening, whitish yellow fluorescent, unbonate, secondary colonies begin to develop along the margin.
3	Yeast extract mannitol agar	Growth abundant, colonies were filiform, citron yellow, fluorescent raised circular entire margin, and secondary colonies begin to develop along the margin.
4	Glucose chalk agar medium	Growth fairly good, filiform slightly raised with entire margin, glistening, pale yellow, fluorescent slightly turn brown.

Abundant growth, filmform nature, glisterning, butyrous, pale yellow fluorescent colonies, observed on nutrient agar medium *in vitro.* Fairly good growth, filiform, slightly raised, glistening, whitish yellow fluorescent,

unbanate, secondary colonies observed on PDA medium. Also filiform, glilstening, yellowish fluorescent filiform, fairly good growth observed on chalk agar medium *in vitro* study.

Table 4

Growth and cultural characters of Pseudomonas syringae pv. gycinea on different agar slants

Sr. No.	Medium	Growth characters after 48 hrs of incubation
1	Nutrient agar	Colonies of first were filiform and light yellow fluorescent then becoming waxy yellow, growth was abundant
2	Potato dextrose agar	Colonies at first were light yellow, fluorescent filirom, unbanate good growth.
3	Yeast extract mannitol agar	Colonies were filiform, citron yellow, fluorescent growth was abundant.
4	Glucose chalk Agar Medium	Colonies were filiform, slight brown colour, growth was abundant

Table 5

Growth and cultural characters of the *Pseudomonas syringae* pv. *glycinea* in different broth.

Sr. No.	Name of broth	Surface growth	Turbidity	Amount of growth
1	Nutrient broth	Slight growth, slightly fluorescent	Light cloudy	Poor
2	Potato dextrose broth	Membranous	Light cloudy	Scanty
3	Yeast extract mannitol agar broth	Pellicle	Light cloudy	Moderate
4	Glucose chalk Agar Broth	Pellicle	Light cloudy	moderate

The symptoms typically resembled with those previously reported by Nishiyama et *al* (1986), Verma (1995) and Srinivasan (1994). Reisolation studies revealed the presence of the same fungus identical to the original one obtained from naturally bacterial blight plants. The morphological and cultural characteristics of the *P. syringae* obtained after reisolation were similar to those reported earlier by several workers (Akhtar and Khan, 1988; Li young Hao *et al.,* (1995) and Supriadi *et al.,* (1996). Two weeks after germination leaves of seedling in each pot were smeared with bacterial suspension by means of sterile cotton incubation period, typical small yellow spots on leaves were observed. Young leaves are most susceptible to the bacterial infection; therefore lesions are small yellow to brown spots on leave. The lesions dry out, turn reddish brown to black and become surrounded by a yellowish green halo. The small lesions may enlarge and merge to produce large, irregular, dead areas. The old lesions sometimes drop out or tear away, resulting in ragged appearance of infected leaves. The bacteria can also infect stem petioles and pods. Highest disease incidence and severity noticed in Parbhani district followed by Hingoli, Nanded, Latur and Beed while lowest disease incidence noticed in Jalna district. The cultural studies results obtained are in agreement with the findings of Song Shuyun *et al.,* 2002.

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