The Bioscan 10(4): 1475-1481, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

STANDARDIZATION OF FIELD INOCULATION TECHNIQUES FOR PROGRESSION OF BACTERIAL BLIGHT OF POMEGRANATE IN PUNJAB

ASHISH AND ANITA ARORA*
Department of Fruit Science,
Punjab Agricultural University, Ludhiana -141 004, Punjab
e-mail: anitapau@pau.edu

KEYWORDS
Bacterial blight
Pomegranate
Xanthomonas axonopodis pv. *punicae*
Inoculation techniques
Infection rate
AUDPC

Received on :
10.06.2015

Accepted on :
18.10.2015

***Corresponding author**

ABSTRACT
Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is a wide spread disease affecting its successful production and every year results into 50-100 per cent economic losses depending upon disease severity. Therefore, field experiment was conducted to standardize the field inoculation techniques for progression of bacterial blight. Inoculations were carried out on leaves, twigs, flowers and fruits of Ganesh and Kandhari variety, under field conditions in the month of June, July and August 2013. For inoculations on different plant parts, different methods were used. It was observed that per cent index of bacterial blight was high, when inoculations were carried out in the month of July than in the months of June and August. High disease index was recorded on leaves (73.50 and 71.50 per cent on Ganesh and Kandhari, respectively) and fruit surface (79.50 and 75.50 per cent on Ganesh and Kandhari, respectively). The degree of infection was varied on the inoculated fruit surface (0.161 and 0.143 per unit per day on Ganesh and Kandhari, respectively) and leaves (0.118 and 0.112 per unit per day on Ganesh and Kandhari, respectively), the former showing high level of disease occurrence. Leaf-clip and pinprick method on fruit surface were selected as the suitable inoculation techniques for identifying resistant genotypes of pomegranate against bacterial blight.

INTRODUCTION

Pomegranate (*Punica granatum* Linnaeus.) called as "Fruit of paradise" is an ancient fruit belonging to the smallest botanical family Punicaceae. In India, pomegranate crop is being cultivated over an area of 112.74 thousand hectare with production of 741.08 thousand MT in 2012-13 (Anonymous, 2013). Pomegranate is common to the tropics, sub-tropics and sub-temperate regions and is well adapted to areas with hot and dry summers.

Pomegranate has renewed interest as a commercial orchard crop because of the health benefits associated with its high level of antioxidants in the pulp or juice. The fresh juice contains moisture, total sugar, pectin, carbohydrate, acidity (as citric acid), minerals like calcium, phosphorus, iron, magnesium and vitamins (Dutta ray *et al.,* 2014). The flower buds are very useful in Ayurveda for managing bronchitis. The bark of the stem, root and rind of fruit is used for slimming, control of dysentery, diarrhoea and killing of tape worms. The bark is also used in tanning industry (Patil and Karle, 1990). However, pomegranate cultivation is facing several constraints and among them bacterial blight is a major tailback in its successful cultivation. The disease was firstly reported in India from Delhi in 1952 by Hingorani and Mehta (1952) and later from Bangalore (Karnataka) in 1959 (Hingorani and Singh, 1959). Chand and Kishun (1991) noticed the epidemics of this disease causing 60-80 per cent losses at IIHR experimental plots in Bangalore. Since 2002, the disease has reached the alarming stage and hampering the Indian economy vis-à-vis export of quality fruits. Outbreak of the disease was noticed in major pomegranate growing areas of Bellary and Bijapur districts causing severe loss both in terms of yield and quality in 2002 (Anonymous, 2002). The disease accounted up to 70–100 per cent during 2006 in Karnataka and Maharashtra resulting in wipe out of pomegranate. During 2007, the total output of pomegranate production in India was down by 60 per cent (Raghavan, 2007). Raghuwanshi *et al.,* (2013) observed 20-88 per cent average prevalence of bacterial blight in four major pomegranate growing districts of Western Maharashtra.

Field observations have revealed bacterial blight as serious threat to cultivation of recommended varieties of pomegranate in Punjab. Disease index varied from 18.43 to 45.67 per cent on fruits and 15.32 to 37.81 per cent on leaves of different cultivars in Punjab (Rani, 1998). Of the several disease management strategies, varietal resistance is considered as the best alternative. This management strategy is possible through standardization of field inoculation methods (Kanwar, 1976, Chand and Kishun, 1991 and Mogle *et al.,* 2009). Considering the above, in the present investigation, efforts have been made to standardize the field inoculation techniques for progression of bacterial blight of pomegranate and to select the suitable inoculation technique for screening

of pomegranate genotypes.

MATERIALS AND METHODS

Isolation of the pathogen

The pathogen was isolated from diseased samples by adapting streak plate method (Hayward, 1983). The bacterial culture was purified on nutrient agar medium and preserved by storing polypropylene tubes having bacterial cells along with silica gel at -80ºC.

Preparation of bacterial suspension

For preparation of bacterial suspension, viable plate count method was used. Firstly bacterial culture was transferred to Peptone Sucrose Broth (PSB) medium in tubes. These tubes were kept in incubator cum shaker at 28ºC for 24 hours. One ml of bacterial culture was picked with the help of pipette and transferred to blank tubes having 9 ml sterile distilled water. Serial dilution method was used upto 6 fold. At 10^{-6} dilution, 100 µl bacterial suspension was picked with micro pipette and transferred to Petriplates having agar media. The suspension was spread with spreader and kept at 28ºC for 24 hours. Colonies were counted with the help of software. Firstly took the photograph of the Petriplate having bacterial colonies using the software Gene Snap. After that this photograph was checked under the wavelength of 900 nm using the software Snap tools.

Artificial inoculations

Inoculations were carried out on leaves, twigs, flowers and fruits under field conditions in months of June, July and August using 1x 10^8 cfu ml^{-1} bacterial suspension of 24 hours old bacterial culture. The varieties Ganesh and Kandhari were selected in the orchard for doing the artificial inoculations. Different inoculation methods were used as standardized by Kanwar (1976), Chand and Kishun (1991), Manjula (2002) and Mogle *et al.* (2009).

Inoculations on leaves

The leaves were firstly washed with sterile distilled water and then inoculated by leaf-clip method in which incision to the intact leaf was given near the tip with sterile scissors dipped in 24 hours old culture suspension. Inoculated leaves were covered with polythene bags containing a wet cotton swab and tied firmly to the twig.

Inoculations on twigs

The twigs were washed with sterile distilled water and inoculated by spray method with the help of hand sprayer. Inoculated twigs were covered with polythene bags containing a wet cotton swab and tied firmly to the twig.

Inoculations on flowers

Healthy full-grown intact flowers were marked on the branches in the orchard. The surface of the flowers was thoroughly washed with sterile distilled water and sterilized with cotton swab dipped in absolute alcohol. The inoculations were carried out on flowers by spray method with the help of hand sprayer. Inoculated flowers were covered with polythene bags containing a wet cotton swab and tied firmly to the twig.

Inoculations on fruits

Healthy medium size intact fruits were marked on the branches in the orchard. The surface of fruits was thoroughly washed with sterile distilled water and sterilized with cotton swab dipped in absolute alcohol. The inoculations were carried out on fruits by pinprick methods:

Pinprick method (Fruit surface)

Selected fruits were inoculated by pins on the fruit surface. The surface was injured by giving shallow pin pricks in the marked areas. Inoculum suspension was prepared from 24 hours old bacterial culture. Small suspension drops were placed on the injured surface with a sterile needle. The inoculated fruits were covered by polythene bags containing a wet cotton swab and tied firmly to the twig.

Pinprick method (Stylar end)

Selected fruits were inoculated by pins on stylar end of the fruit. Inoculum suspension was prepared from 24 hours old bacterial culture. Small suspension drops were placed on the injured stylar end with a sterile needle. The inoculated fruits were covered by polythene bags containing a wet cotton swab and tied firmly to the twig.

The following observations were recorded:

(i) Per cent disease index was recorded at weekly interval after inoculations.

Per cent Disease Index was determined by using the following formula (McKinney, 1923):

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves/fruits/twings/ flowers examined} \times \text{maximum rating}} \times 100$$

A scale (0-4) with index on leaves, twigs, flowers and fruits (Chester, 1950) were adopted for calculating per cent disease index where, 0 = No spot visible on the leaves, 1 = One-fourth of the leaf area spotted, 2 = Half of the leaf area spotted, 3 = Three- fourth of the leaf area spotted and 4 = More than three-fourth of the leaf area spotted.

(ii) Infection rate (Vander plank, 1963) was calculated using the following formula:

$$r = \frac{2.3}{t_2 - t_1} \times \log_e\left(\frac{x_2}{1-x_2}\right) - \log_e\left(\frac{x_1}{1-x_1}\right)$$

Where, r is the infection rate, t_1 is the time of the first measurement, t_2 is the time of the second measurement, x_1 is the proportion of infection measured at time t_1 and x_2 is the proportion of infection measured at time t_2.

(iii) Area Under Disease Progress Curve (AUDPC) value (A-value) was calculated by equation given by Wilcoxson *et al.*, (1975).

$$\text{A-value} = \sum_{i=1}^{k} 1/2(S_i + S_{i-1}) \times d$$

Where,

S_i = Disease severity at every 7th day

k = The number of successive evaluation of disease

d = Interval between two evaluations

RESULTS AND DISCUSSION

Artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* on Ganesh variety

Per cent disease index

It is evident from the data presented in Table 1 that when inoculations were done in the months of June, July and August, per cent disease index progressed gradually and became maximum after 28 days of inoculations. Typical black spots appeared on leaves, twigs, flowers and fruits within 4-7 days of inoculations. These findings are in conformity with those of Rangaswami (1962), Kanwar (1976), Chand and Kishan (1991), Kishun (1993), Rani (1998) and Manjula and Khan (2002). Kanwar (1976) observed the symptoms within four to seven days on injured portions and it took 8-10 days to get the symptoms on uninjured parts. The characteristic symptoms were observed on leaves as small, water-soaked, brown to black coloured lesions, which later on developed into angular to irregular shaped spots along the veins and veinlets of the leaf lamina leading to marginal necrosis. The initial symptoms of the disease on the developing flowers were characterized by production of minute, water-soaked spots with conspicuously depressed surface. These spots increased in size, became irregular and turned brown and necrotic. On the fruits, symptoms were noticed as small, pin head sized, black lesions with diffused water-soaked margins, which later on, developed into black coloured, large sized spots. Similar symptomatological detail of the disease was also obtained by Chand and Kishun (1991), Rani (1998), Jadhav and Sharma (2009) and Jamadar *et al.,* (2009) who noticed small, water-soaked, brown to dark brown spots on leaves and oily, dark brown to black spots with L or Y shaped cracks on fruits. Similarly, irregular, water-soaked spots (2 to 5 mm diameter) on foliage were observed by Mulla *et al.,* (2009) and Mondal *et al.,* (2012).

It was observed that per cent disease index increased gradually from 17.50 to 70.50 on leaves, 8.50 to 43.50 on twigs and 10.50 to 51.50 on flowers in Ganesh variety when inoculations were done in June (Table 1). Maximum disease index was recorded on the inoculated fruit surface. The disease index recorded on the inoculated fruit surface was increased from 18.50 to 72.00 per cent. Whereas increase in per cent disease index on the fruits inoculated on stylar end was from 13.50 to 63.50. When the plants were inoculated in July, per cent disease index increased from 20.50 to 73.50 on leaves, 12.50 to 52.50 on twigs, 15.50 to 60.00 on flowers, 25.50 to 79.50 on the fruits inoculated on its surface and 18.50 to 69.50 on the fruits inoculated on stylar end. When inoculations were carried out in August, it was observed that per cent disease index increased from 16.50 to 68.50 on leaves, 12.50 to 46.50 on twigs, 10.50 to 48.50 on flowers, 18.50 to 71.50 on the fruits inoculated on its surface and 14.50 to 62.50 on the fruits inoculated on stylar end. The data were also illustrated in Fig. 1.

The results clearly revealed that per cent disease index was high, when inoculations were carried out in the month of July than in the months of June and August. The pathogen successfully established on all the inoculated plant parts viz. leaves, flowers, twigs and fruits of Ganesh variety. High disease index was recorded on leaves and fruits as compared to flowers and twigs. It might be due to the factor that the pathogen preferred the injury provided by clip method on leaves and pinprick method on fruits. These observations had confirmed the earlier findings of Hingorani and Mehta (1952), Kanwar

Table 1: Development of bacterial blight on different plant parts of pomegranate variety Ganesh under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* during 2013.

Date of inoculation	Observation (Days after inoculation)	Per cent disease index				
		Leaves	Twigs	Flowers	Fruits	
					Fruit surface	Stylar end
17-June-2013	7	17.50	8.50	10.50	18.50	13.50
	14	24.50	12.50	15.50	27.00	19.50
	21	43.33	24.33	29.50	49.33	36.50
	28	70.50	43.50	51.50	72.00	63.50
08-July-2013	7	20.50	12.50	15.50	25.50	18.50
	14	29.50	17.50	22.00	36.50	26.00
	21	48.50	30.50	37.50	62.00	43.50
	28	73.50	52.50	60.00	79.50	69.50
05-August-2013	7	16.50	12.50	10.50	18.50	14.50
	14	27.50	18.75	17.50	31.00	24.50
	21	44.50	29.33	30.50	50.50	39.50
	28	68.50	46.50	48.50	71.50	62.50

Table 2: Infection rate of bacterial blight on different plant parts of pomegranate variety Ganesh under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* during 2013.

Month of inoculation	Infection rate (per unit per day) (r)				
	Leaves	Twigs	Flowers	Fruit Surface	Stylar end of fruit
June, 2013	0.113	0.085	0.096	0.116	0.104
July, 2013	0.118	0.099	0.101	0.161	0.113
August, 2013	0.115	0.096	0.099	0.127	0.111

Table 3: Area Under Disease Progress Curve (AUDPC) of bacterial blight on different plant parts of pomegranate variety Ganesh under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* during 2013.

Months of inoculation	DAI*	Leaves	Twigs	Flowers	Fruits	
					Fruit surface	Stylar end of fruit
June,2013	14	147.00	73.50	91.00	159.25	115.50
	21	237.41	128.91	157.50	267.16	196.00
	28	398.41	237.41	283.50	424.66	350.00
July,2013	14	175.00	105.00	131.25	217.00	155.75
	21	273.00	168.00	208.25	344.75	243.25
	28	427.00	290.50	341.25	495.25	395.50
August,2013	14	154.00	109.38	98.00	173.25	136.50
	21	252.00	168.28	168.00	285.25	224.00
	28	395.50	265.41	276.50	427.00	357.00

DAI*- Days After Inoculation

Table 4: Development of bacterial blight on different plant parts of pomegranate variety Kandhari under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* during 2013

Date ofInoculation	Observation (Days after inoculation)	Per cent disease index				
		Leaves	Twigs	Flowers	Fruits	
					Fruit surface	Stylar end
17-June-2013	7	15.50	4.50	7.50	15.33	12.50
	14	21.50	6.50	10.50	23.33	17.50
	21	36.00	10.50	18.50	39.50	30.00
	28	55.00	19.00	32.50	61.50	48.00
08-July-2013	7	21.50	12.00	17.50	20.50	20.00
	14	33.33	16.80	24.50	31.00	29.00
	21	55.80	26.50	36.50	53.50	45.00
	28	71.50	43.50	55.50	75.50	68.00
05-August-2013	7	15.50	11.50	9.00	17.50	12.50
	14	27.50	15.50	13.50	28.50	20.50
	21	42.50	23.50	21.80	45.50	40.00
	28	65.50	34.50	36.50	69.50	60.50

Table 5: Infection rate of bacterial blight on different plant parts of pomegranate variety Kandhari under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* during 2013

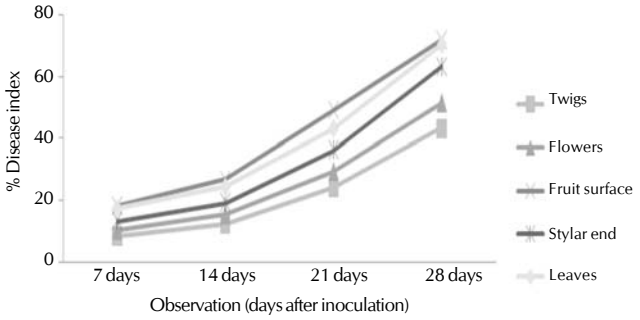
Month of inoculation	Infection rate (per unit per day) (r)				
	Leaves	Twigs	Flowers	Fruit Surface	Stylar end of fruit
June, 2013	0.090	0.073	0.083	0.103	0.088
July, 2013	0.112	0.082	0.085	0.143	0.096
August, 2013	0.105	0.076	0.084	0.113	0.090

Table 6: AUDPC of bacterial blight on different plant parts of pomegranate variety Kandhari under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* during 2013.

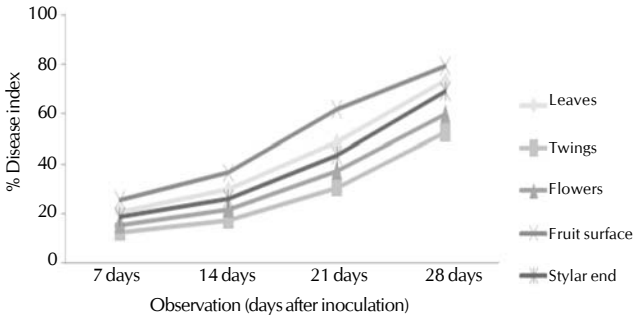
Months of inoculation	DAI*	Leaves	Twigs	Flowers	Fruits	
					Fruit surface	Stylar end of fruit
June,2013	14	129.50	38.50	63.00	135.31	105.00
	21	201.25	59.50	101.50	219.90	166.25
	28	318.50	103.25	178.50	353.50	273.00
July,2013	14	191.91	100.80	147.00	180.25	171.50
	21	311.96	151.55	213.50	295.75	259.00
	28	445.55	245.00	322.00	465.50	395.50
August,2013	14	150.50	94.50	78.75	161.00	115.50
	21	245.00	136.50	123.55	259.00	211.75
	28	378.00	203.00	204.05	402.50	351.75

DAI*- Days After Inoculation

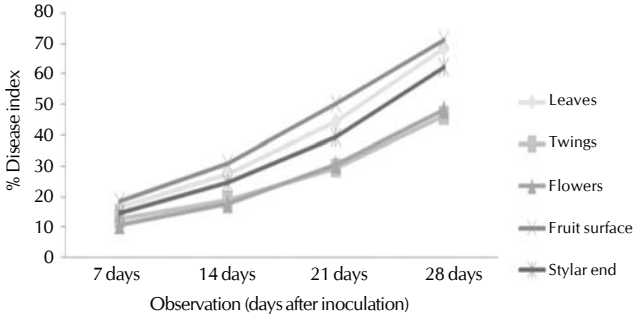
(1976), Chand and Kishun (1991), Manjula (2002), Vernière *et al.,* (2003), Yenjerappa (2009) and Mogle *et al.,* (2009). Hingorani and Mehta (1952) used spray method of inoculation in absence of wounds. Infection occurred on the tender leaves of artificially inoculated young potted plants within 7 - 10 days after incubation. Kanwar (1976) inoculated leaves, flowers



a) Inoculations in the month of June

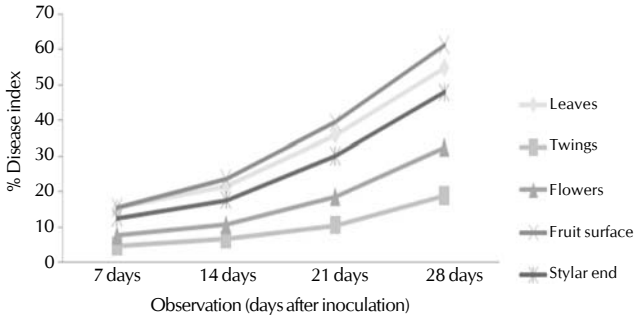


b) Inoculations in the month of July

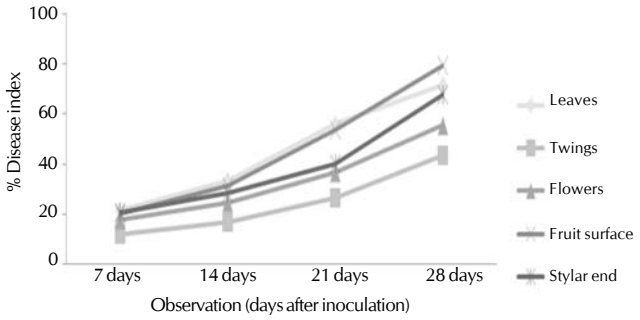


c) Inoculations in the month of August

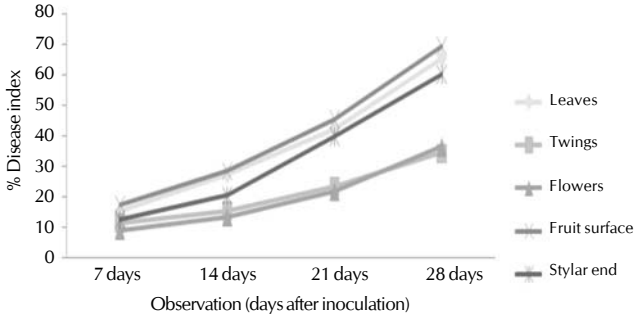
Figure 1(a-c): Development of bacterial blight on different plant parts of pomegranate variety Ganesh under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punica*e during 2013



a) Inoculations in the month of June



b) Inoculations in the month of July



c) Inoculations in the month of August

Figure 2(a-c): Development of bacterial blight on different plant parts of pomegranate variety Kandhari under periodical artificial inoculations of *Xanthomonas axonopodis* pv. *punica*e during 2013

and fruits of healthy plants by spraying bacterial suspension onto the injured and uninjured plant parts. Infection occurred more rapidly on injured leaves, flowers and fruits than uninjured. Chand and Kishun (1991) standardized the inoculation method by inoculating the bacterial suspension on leaves by pinprick, rubber block pressure, leaf cut and automization methods. They observed that, leaf cut was found superior, where they recorded 100 per cent infection covering 70 to 90 per cent leaf area within 21 days. The automization of bacterial suspension was found to induce lowest infection of 6.0 to 7.5 per cent with maximum incubation period of 17 to 40 days. Similarly, Vernière *et al*. (2003) reported that susceptibility of the fruit increased with pinprick method of inoculation. Mogle *et al*. (2009) inoculated highly susceptible cultivar Ganesh by leaf cut method and recorded that typical symptoms of the disease appeared on leaves within 9 to 13 days after inoculation.

Infection rate and Area Under Disease Progress Curve (AUDPC)

The data presented in Table 2 showed that with increase in time, infection rate of bacterial blight was increased on Ganesh variety of pomegranate. The degree of infection was varied on inoculated fruit surface and leaves, the former showing high level of disease occurrence. It was also observed that infection rate of the disease was highest on the inoculated fruit surface, when inoculations were carried out in the month of July than in the months of June and August.

From the results given in Table 3 it is evident that AUDPC was maximum in the month of July than in the months of June and August. AUDPC was maximum (495.25) on fruits when inoculated on fruit surface followed by leaves (427.00), when inoculated with clip method in the month of July. It was observed that value of AUDPC increased with increase in degrees of susceptibility which was in agreement with the results of Gottwald *et al*. (1989) and Vernière *et al*. (2003).

Artificial inoculations of *Xanthomonas axonopodis* pv. *punicae* on Kandhari variety

Similar trend was observed when different plant parts of pomegranate variety Kandhari (Table 4 and Fig. 2) were inoculated with suspension of *Xanthomonas axonopodis* pv. *punicae* in the months of June, July and August. The data indicated that variety Ganesh was more susceptible than Kandhari. Therefore, Infection rate of bacterial blight and AUDPC were much higher in Ganesh as compared to Kandhari (Table 5 and 6), thereby, showing the fast development of the disease in Ganesh than that in Kandhari. These findings are in conformity with those of Jalikop *et al*. (2006) and Mogle *et al*. (2009) who categorized Ganesh as highly susceptible cultivar.

The present investigation clearly indicated that per cent disease index, infection rate and AUDPC of bacterial blight were maximum, when inoculations on leaves and fruits were carried out by leaf- clip and pinprick method, respectively. Therefore, leaf-clip and pinprick method on fruit surface were selected as the suitable inoculation techniques for identifying resistant genotypes of pomegranate against bacterial blight. The results also revealed that July was the most favourable month for bacterial blight development. Thus, these studies can be utilized further for proper management of bacterial blight of pomegranate.

REFERENCES

Anonymous, 2002. Pomegranate growers are in dire straits. *The Hindu*, p. 8.

Anonymous, 2013. Area and Production of pomegranate fruit in India. *Indian Horticulture Database*. http://www.nhb.gov.in/.

Chand, R. and Kishun, R. 1991. Studies on bacterial blight (*Xanthomonas campestris* pv. *punicae*) of pomegranate. *Indian Phytopathol*. **44**: 370-371.

Chester, K. 1950. Plant disease losses: their appraisal and interpretation. *Plant Dis. Reptr.* **193(Supple):** 189-362.

Dutta Ray, S. K., Takawale, P. V., Chatterjee, R. and Hnamte, V. 2014. Yield and quality of pomegranate as influenced by organic and inorganic nutrients. *The Bioscan*. **9**: 617-620.

Giri, M. S. 2009. Morphological and molecular variability of *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vauterin *et al.,* causing bacterial blight of pomegranate. *M. Sc. (Agri.) Thesis*, Univ Agric Sci. Bangalore (India).

Gottwald, T. R., Timmer, L. W., and McGuire, R. G. 1989. Analysis of disease progress of citrus canker in nurseries in Argentina. *Phytopathology*. **79**:1276-1283.

Hayward, A. C. 1983. Preliminary diagnosis of plant diseases: a diagnostic guide. In: Fahy PC & Persley GJ (Eds) Plant Bacterial Diseases: A Diagnostic Guide Academic Press, NSW, Australia. pp. 1-12.

Hingorani, M. K. and Mehta, P. P. 1952. Bacterial leaf spot of pomegranate. *Indian Phytopathol.* **5**:55-56.

Hingorani, M. K. and Singh, N. J. 1959. *Xanthomonas punicae* sp. Nov. on pomegranate (*Punica granatum* L.). *Indian J. Agric. Sci.* **29**:45-48.

Jadhav, V. T. and Sharma, K. K. 2009. Integrated management of diseases in pomegranate. Paper Presented In: *2nd Inter Symp Pomegranate and minor including Mediterranean Fruits,* Univ. Agric. Sci., Dharwad, June 23-27, pp. 48-52.

Jalikop, S. H., Kumar, P. S., Rawal, R. D., and Kumar, R. 2006. Breeding pomegranate for fruit attributes and resistance to bacterial blight. *Indian J. Hort.* **63**:352-358.

Jamadar, M. M., Jawadagi, R. S., Sataraddi, A. R., Patil, D. R. and Patil, P. V. 2009. Status of pomegranate diseases and their management in Northern Karnataka in India. Paper Presented In: *2nd Inter Symp Pomegranate and minor including Mediterranean Fruits,* Univ. Agric. Sci., Dharwad, June 23-27, pp. 134.

Kanwar, Z. S. 1976. A note on bacterial disease of pomegranate (*Punica granatum* L.) in Haryana. *Haryana J. Hort. Sci.* **5**:177-180.

Kishun, R. 1993. Bacterial disease of fruits. In : *Advances in Horticulture*, Vol. 3 (Eds.) : Chand, K. L. and Pareek, O. P., Malhotra Publishing House, New Delhi, pp. 1398-1400.

Manjula, C. P. 2002. Studies on bacterial blight of pomegranate (*Punica granatum* L.) caused by *Xanthomonas axonopodis* pv. *punicae*. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Bangalore, Karnataka (India).

Manjula, C. P. and Khan. A. N. A. 2002. Incidence of bacterial blight of pomegranate (*Punica granatum* L.) in Karnataka. Paper presented at the *Annu. Meet. Symp. Plant Disease Scenario in Southern India*, held at Bangalore (India) on December 19-21, p. 51-52.

Mckinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helmintosporium sativum. J. Agric. Res.* **26**:195-217.

Mogle, T. R., Magar, S. J., Suryawanshi, A. P. and Somwanshi, S. D. 2009. Pathogenicity, morphology and cultural characterstics of *Xanthomonas axonopodis* pv. *punicae*, causing oily spot of pomegranate. Paper Presented In: *2nd Inter Symp Pomegranate and minor including Mediterranean Fruits,* Univ. Agric. Sci., Dharwad, June 23-27, pp. 137.

Mondal, K. K., Rajendran, T. P., Phaneendra, C., Mani, C., Sharma, J., Shukla, R., Pooja., Verma. G., Kumar, R., Singh, D., Kumar, A., Saxena, A. K. and Jain, R. K. 2012. The reliable and rapid polymerase chain reaction (PCR) diagnosis for *Xanthomonas axonopodis* pv. *punicae* in pomegranate. *African J. Microbiol. Res.* **6**:5950-5956.

Mulla, S. M., Suryawanshi, A. P., Dhutraj, D. N. and Magar, S. J. 2009. Survey and Surveillance of bacterial blight (oily spot) disease of pomegranate in Maharashtra, India. Paper Presented In: *2nd Inter Symp Pomegranate and minor including Mediterranean Fruits,* Univ. Agric. Sci., Dharwad, June 23-27, pp. 136.

Patil, A. V. and Karle, A. R. 1990. Pomegranate In: Fruits, tropical and sub-tropical, Ed: Bose, T. K. and Mitra, S. K., Naya Prakash Publications, Calcutta, pp. 616-34.

Raghavan, R. 2007. Oily spot of pomegranate in India (Maharashtra), *Express India,* dated: 18.11.2007.

Raghuwanshi, K.S., Hujare, B.A., Chimote V.P. and Borkar, S.G. 2013. Characterization of *Xanthomonas axonopodis* pv. *punicae* isolates from Western Maharashtra and their sensitivity to chemical treatments. *The Bioscan*.**8**: 845-850.

Rangaswami, G. 1962. Pomegranate. In: *Bacterial plant disease in India*. Asia publication house, Bombay, pp. 830.

Rani, U. 1998. Investigations on black spot of pomegranate (*Punica granatum* L.) and its management. *M. Sc. (Agri.) Thesis*, P.A.U., Ludhiana, Punjab (India).

Vander Plank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York.

Vernière, C. J, Gottwald, T. R., and Pruvost, O. 2003. Disease development and symptom expression of *Xanthomonas axonopodis* pv. *citri* in various citrus plant tissues. *Phytopathol*. **93**:832-843.

Wilcoxson, R. D., Skovmand, B. and Atif, A. H. 1975. Evaluation of wheat cultivars for their ability to retard development of stem rust. *Ann. Appl. Biol.* **80**: 275-82.

Yenjerappa, S. T. 2009. Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punica*e (Hingorani and Singh) *Ph. D. Thesis*, Univ. Agri. Sci. Dharwad (India).