

# INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Research Article.....!!!

Received: 24-09-2014; Revised; Accepted: 07-10-2014

## EFFECT OF PHYSICAL FACTORS ON *XANTHOMONAS AXONOPODIS* PV. *PUNICAE*

V. B. Chopade\*<sup>1</sup>, S. D. Shaikh<sup>3</sup> and S. S. Kamble<sup>2</sup>

1. Department of Botany, Rajarshi Chhatrapati Shahu College, Kolhapur
2. Department of Botany, Shivaji University, Kolhapur.
3. Department of Botany, Abasaheb Marathe Arts & New Commerce, Science College, Rajapur, India

### Keywords:

Pomegranate,  
*Xanthomonas*, effect of  
pH and temperature

### For Correspondence:

**V. B. Chopade**  
Department of Botany,  
Rajarshi Chhatrapati Shahu  
College, Kolhapur

### E-mail:

[ranichopade26@gmail.com](mailto:ranichopade26@gmail.com)

### ABSTRACT

Maharashtra produces about 17.54 m. MT of horticultural produce from an area of 2.49 m. ha. accounting for 7.30% of horticulture production in the country. It is the leading producer of Pomegranate in the country and accounts for 66.2% of the total production of pomegranate in the country. Large scale infestation of Bacterial Blight Disease caused due to *Xanthomonas axonopodis* pv. *punicae* has resulted in considerable damage to the crop from 2006-07. Studies on morphological, biochemical and physiological features of the pathogen are of immense use in understanding the nature of the pathogen. The studies will help in the management of the disease.

## INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae. It is an ancient fruit crop of India. The fruits of pomegranate are known to possess pharmaceutical and therapeutic properties with high medicinal value. The bark is also used in tanning industry<sup>1</sup>. Among the diseases infecting pomegranate, the bacterial disease popularly known as ‘bacterial blight’ caused by *Xanthomonas axonopodis* pv. *Punicae*<sup>2</sup>. Pomegranate “the boon commercial fruit crop to the farmer turned as a big bane after the severe outbreak of bacterial blight. Many growers finding no options to mitigate the disease effectively have uprooted their crop owing to unbearable losses.

Studies on morphological, biochemical and physiological features of the pathogen are of immense use in understanding the nature of the pathogen. *Xanthomonas axonopodis* pv. *punicae* bacterium shows optimum growth at temperature 30°C and pH 7.0.

## MATERIAL AND METHOD

Among the fourteen isolates of *Xanthomonas axonopodis* pv. *punicae*., most sensitive and resistant against streptomycin were derived and used to study the effect of physical factors on it.

**Effect of pH:** Both the sensitive (Xap-01) and resistant (Xap-11) isolates were cultured in nutrient glucose broth medium at various pH values. The pH was adjusted with 1N HCL and 1N NaOH. The sensitive and resistant isolates were grown in medium at different pH levels. The inoculated test tubes in triplicate at different pH were incubated at 30°C and growth was recorded turbidometrically after 24 hrs incubation using Shimadzu, UV-VIS, 1800 double beam spectrophotometer at 660 nm.

**Effect of temperature:** To study effect of temperature *in vitro* the sensitive (Xap-01) and resistant (Xap-11) isolates inoculated in test tubes containing nutrient glucose broth medium and kept at different temperature in BOD incubator. Test tubes at room temperature served as control. Growth was recorded turbidometrically after 24 hrs incubation using Shimadzu, UV-VIS, 1800 double beam spectrophotometer at 660 nm.

To study effect of temperature *in vivo*, the healthy pomegranate fruits were inoculated with bacterial suspension. For this on fruits 6 mm diameter and 15 mm deep well was prepared with the help of sterile cork borer and inoculated with sensitive (Xap-01) and resistant (Xap-11) isolate solutions individually. Inoculated pomegranate fruits were wrapped with sterilized paper and percentage (%) infection was recorded at every day upto four days of incubation periods. Fruits at room temperature served as control.

## RESULT

### Effect of p<sup>H</sup>

*Xanthomonas axonopodis* pv. *punicae* sensitive (Xap-01) and resistant (Xap-11) isolates were cultured in nutrient glucose broth medium at various p<sup>H</sup> levels. The inoculated test tubes at different p<sup>H</sup> were incubated at 30<sup>0</sup>C for 24 hours. It was seen from (Table. 1) that the growth of resistant isolate Xap-11 was higher than that of sensitive isolate Xap-01. p<sup>H</sup> 7.0 was most favourable for the sensitive and resistant isolates of *Xanthomonas axonopodis* pv. *punicae*.

### Effect of Temperature

#### *In vitro* studies

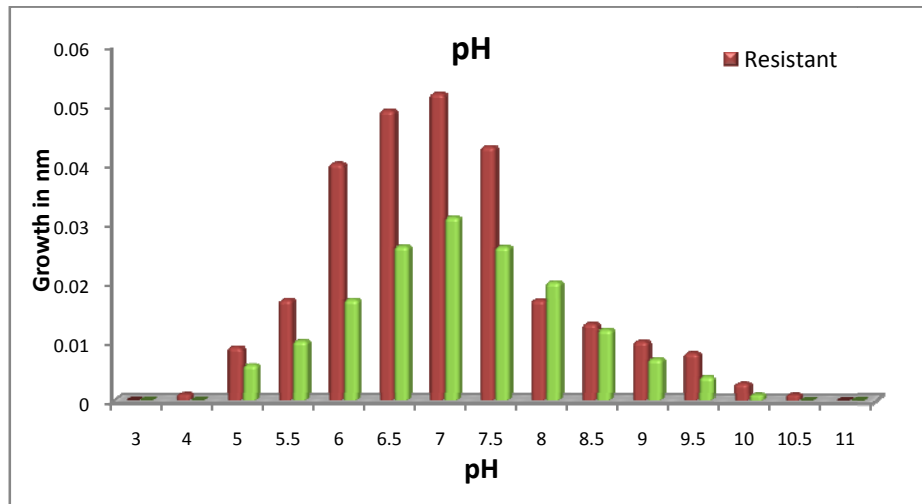
For temperature effect the tubes with NG broth medium were inoculated with sensitive and resistant isolates were kept at different temperature in BOD incubator. Tubes at room temperature served as control. Growth was measured after 24 hours. It was seen that all temperature there was higher growth of resistant isolate. At 30<sup>0</sup>C the isolates showed their maximum growth. Both sensitive (Xap-01) and resistant (Xap-11) isolates were failed to grow at low temperature (10<sup>0</sup>C and 15<sup>0</sup>C). (Table. 2).

#### *In vivo* studies

To study effect of temperature *in vivo*, the healthy pomegranate fruits are inoculated with sensitive and resistant isolates and kept at different temperature in BOD incubator. Fruits at room temperature served as control. Percentage (%) infection was measured every day upto four days. It was seen that all temperature there was higher growth of resistant isolate. At 30<sup>0</sup>C the isolates showed their maximum growth. Both sensitive (Xap-01) and resistant (Xap-11) isolates were failed to grow at low temperature (10<sup>0</sup>C and 15<sup>0</sup>C). (Table. 3).

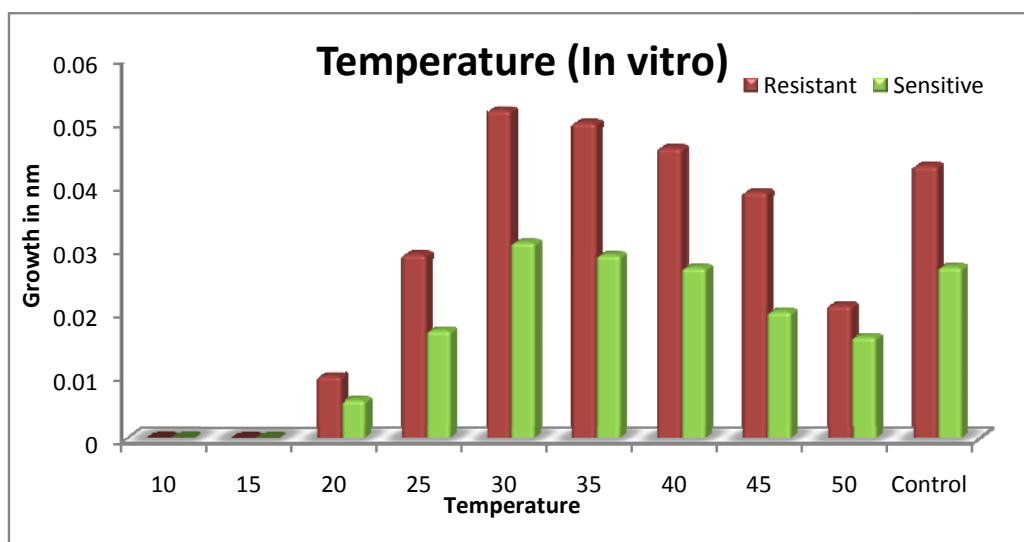
**Table.1: Effect of p<sup>H</sup> on the growth (turbidity at 660 nm) of *Xanthomonas axonopodis* pv. *punicae* isolates in NG broth medium.**

p <sup>H</sup>	Resistant	Sensitive
03	0.000	0.000
04	0.001	0.000
05	0.009	0.006
5.5	0.017	0.010
06	0.040	0.017
6.5	0.049	0.026
07	0.052	0.031
7.5	0.043	0.026
08	0.017	0.020
8.5	0.013	0.012
09	0.010	0.007
9.5	0.008	0.004
10	0.003	0.001
10.5	0.001	0.000
11	0.000	0.000



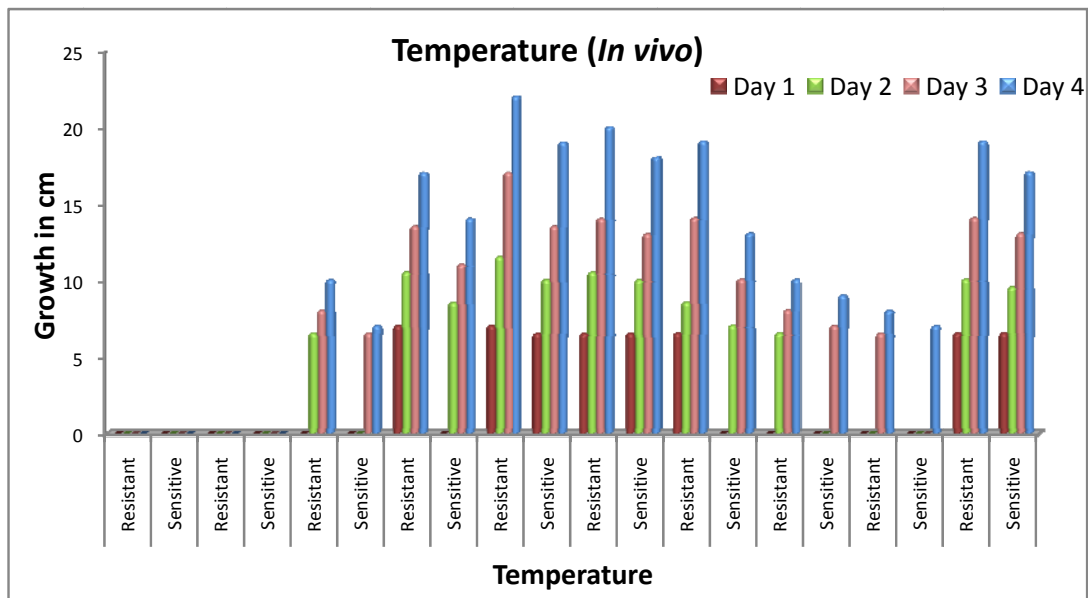
**Table.2: Effect of Temperature on the growth (turbidity at 660 nm) of *Xanthomonas axonopodis* pv. *punicae* isolates in NG broth medium. (*In vitro*)**

Temperature	Resistant	Sensitive
10 <sup>0</sup> C	0.000	0.000
15 <sup>0</sup> C	0.000	0.000
20 <sup>0</sup> C	0.010	0.006
25 <sup>0</sup> C	0.029	0.017
30 <sup>0</sup> C	0.052	0.031
35 <sup>0</sup> C	0.050	0.029
40 <sup>0</sup> C	0.046	0.027
45 <sup>0</sup> C	0.039	0.020
50 <sup>0</sup> C	0.021	0.016
Control	0.043	0.027



**Table.3: Effect of Temperature on the growth (mm) of *Xanthomonas axonopodis* pv. *punicae* isolates. (In vivo)**

Temperature		Days			
		1	2	3	4
10	Resistant	00	00	00	00
	Sensitive	00	00	00	00
15	Resistant	00	00	00	00
	Sensitive	00	00	00	00
20	Resistant	00	6.5	08	10
	Sensitive	00	00	6.5	07
25	Resistant	07	10.5	13.5	17
	Sensitive	00	8.5	11	14
30	Resistant	07	11.5	17	22
	Sensitive	6.5	10	13.5	19
35	Resistant	6.5	10.5	14	20
	Sensitive	6.5	10	13	18
40	Resistant	6.5	8.5	14	19
	Sensitive	00	07	10	13
45	Resistant	00	6.5	08	10
	Sensitive	00	00	07	09
50	Resistant	00	00	6.5	08
	Sensitive	00	00	00	07
Control	Resistant	6.5	10	14	19
	Sensitive	6.5	9.5	13	17



## DISCUSSION

Temperature is an important factor for growth, reproduction and survival of bacterium. In the present study the temperature range of 25<sup>0</sup>C to 30<sup>0</sup>C was found to be optimum for growth of isolates of *Xanthomonas axonopodis* pv. *punicae*. These results are in the linewith work of<sup>3</sup>.

The optimum temperature required for the growth of *Xanthomonas axonopodis* pv. *punicae* required is 30<sup>0</sup>C and it tolerated upto 40<sup>0</sup>C as maximum. Minimum temperature required for the growth is about 5 – 10<sup>0</sup>C<sup>4</sup>. The temperature for the growth of the pathogen *Xanthomonas punicae* sp. was minimum at 5<sup>0</sup>C, optimum at 27<sup>0</sup>-30<sup>0</sup>C and maximum at 40<sup>0</sup>C. They also reported maximum growth of the pathogen at pH 6.8 to 7.6 and no growth at pH 10.2 was also observed<sup>2</sup>. The pathogen, *Xanthomonas axonopodis* pv. *punicae*, developed at wide temperature range of 20 to 40<sup>0</sup>C and documented maximum number of optimum. The bacteria failed to grow at pH 5.0 and 8.0 indicating the narrow range of pH<sup>5</sup>.

## CONCLUSION

Studies on morphological, biochemical and physiological features of the pathogen are of immense use in understanding the nature of the pathogen. The p<sup>H</sup> 7.0 was most favourable for the sensitive and resistant isolates. The isolate grow luxuriantly at 30<sup>0</sup> C but failed at low temperature. The present studies will provide strong management strategies in the bacterial blight disease management of pomegranate.

## ACKNOWLEDGEMENT

Author is thankful to Head Department of Botany, Shivaji University, Kolhapur and Principal, Raj. Chh. Shahu College, Kolhapur for their keen interest and constant encouragement.

## REFERENCES

1. 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. 616-634.
2. HINGORANI, M. K. AND SINGH, N. J., 1959. *Xanthomonas punicae* sp. Nov. on pomegranate (*Punica granatum* L.). *Indian J. Agric. Sci.*, 29:45-48.
3. BHAT, V. V. AND PATEL, M. K., 1954. Comparative study of species of *Xanthomonas* parasitizing leguminous plants in india. *Indian Phytopath.*, 7:160-180.
4. HINGORANI, M. K. AND MEHTA, P. P., 1952. Bacterial leaf spot of pomegranate. *Indian Phytopath.*, 5:55-56.
5. MANJULA, C. P. 2002. Studies on bacterial blight of pomegranate (*Punica granatum* L.) caused by *Xanthomonas axonopodis* pv. *punicae*, M. Sc. (Agri.) Thesis, Univ. of Agric. Sci., Bangalore (India).