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Growth Kinetic Study of Bacterial Pathogens of Pomegranate Crop

Bulbule Vijaykumar Mahadev
Research Scholar, Department of Botany
Lal Bahadur Shastri Mahavidyalaya, Nanded, Maharashtra, India

Abstract: *The activities of microorganisms are greatly affected by the chemical and physical conditions of their environments. Different organisms react to their environment in different ways. An environment that is harmful to one microorganism may be beneficial to another. Sometimes an organism can tolerate an adverse condition in which it is unable to grow. In the present study, optimization of growth conditions of Xanthomonas axonopodis VMB13, Xanthomonas campestris VMB15 and Xanthomonas vesicatoria VMB17 bacterial pathogens of Pomegranate crop were studied. It is observed that the maximum colony number of the pathogens was observed at 30^0C which was followed by 25 ^{0}C, 35^0C. The minimum colony number of the pathogen was observed at 40^0C, 20^0C which was followed by 15^0C. No growth of the pathogens was observed at 10^0C and 50^0C on sterile nutrient glucose agar. The large number of development of colonies was obtained at pH 7.0. Further increase in pH was found to decrease the number of colonies on the agar medium. The maximum number of colonies of the pathogen was observed on agar medium with 0.5% sodium chloride (NaCl) concentration followed by 0.25% and 1.0% salt concentration. Lowest colonies were observed on NG medium with 1.5%, 0.05% sodium chloride concentration respectively.*

Keywords: Kinetic study, Pomegranate Fruit, Temperature, pH, NaCl, etc.

I. INTRODUCTION

Pomegranate is regarded as the “Fruit of Paradise”. It is one of the most adjustable subtropical minor fruit crops and its cultivation is rising very rapidly. The most grown varieties in India are Ganesh, Arakta, Mridula, Ruby, G-137, Bhagawa and Khandar. The area under pomegranate cultivation is increasing worldwide because of its hardy nature, ample adaptability, drought resistance, greater yield, excellent preserving quality and remunerative prices in domestic as well as export markets. It thrives well under hot, dry and cold tropics come up very well in well drained, sandy loam to deep loamy or alluvial soils of low fertility status, adding to that it is salt tolerant too (Shiva Prasad *et al*., 2012).

Plant diseases caused by bacteria and fungi are the major hindrances worldwide in successful cultivation of crops for good yield. There is growing need to control these diseases, so as to ensure continuous supply of food to ever increasing population. The need for increasing agricultural production, productivity and quality has led to excessive use of chemical pesticides, and fungicides creating serious environmental problems. The use of bio-fertilizers and bio-pesticides are some of the alternative approaches for achieving high production with low ecological impact.

Rangaswamy (1962) reported that the bacterium can enter into plant through wounds, stomata openings and caused water soaked lesions with irregular spots on leaves of pomegranate. The bacterium could continue survived for four months in soil spread due to air borne cells. Upasana Rani and Verma (2002) stated that pathogen of bacterial blight of pomegranate survived in fallen infected leaves could remain survived for 210 days. Sheikh (2006) mentioned the bacterium could survive for 120 days on the fallen leaves into soil. The disease spreads through wind splashed rains, use of diseased planting material (mother plant), less spacing between two neighboring plants, equipments used during pruning contaminated with bacterium, during cutting of leaves, different insects, pests, workers and improper sanitation of field itself (Benagi *et al.,* 2012).

The microorganisms respond differently when there is a variation in levels of nutrients. The growth of microorganisms is highly affected by the nature of the surroundings. Microorganisms are ubiquitous; they adapt themselves to survive in unfavorable environments. The microorganisms which thrive and grow in extreme environments where normal microorganisms can’t survive are called extremophiles. The major factors which influence the growth of

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microorganisms are pH, water activity, temperature, oxygen level, pressure, and radiation. These factors help to study the ecological distribution of microorganisms.

In the present study different environmental factors like temperature, pH and sodium chloride (NaCl) concentration were studied on growth of bacterial pathogens *Viz. Xanthomonas axonopodis VMB13, Xanthomonas campestris VMB15* and *Xanthomonas vesicatoria VMB17* of Pomegranate production.

II. MATERIALS AND METHODS

2.1 Effect of Temperature on Growth of the Pathogen

The study was performed to determine the optimum temperature requirement for the growth of the isolated pathogen. The cell density was adjusted to 10^6 -10^7CFU/ml on the basis when culture reaches at 0.1 optical units at 600nm with spectrophotometer (Schaad, 1992). Seventy two hours old bacterial culture (10^6-10^7CFU/ml) was diluted serially using 9ml sterile distilled water blanks. The serial dilutions were prepared from 10^{-1} to 10^{-5} by using sterile distilled water blanks. 100µl from each dilution was taken and spread inoculated on to the sterile nutrient glucose agar medium. Then all the inoculated plates were incubated at different temperature *Viz*.10^{0}C, 20^{0}C, 30^{0}C, 40^{0}C and 50^{0}C for 72 hours. The observations were drawn for the development of colonies on the inoculated plates which were kept at different temperatures for specified time intervals. Number of colonies were counted and recorded (Suresh *et al*., 2013; Yenjerappa, 2009).

2.2 Effect of pH on Growth of the Pathogen

The effect of pH on growth of isolated bacterial and fungal cultures was studied by adjusting the pH of the nutrient glucose medium to different pH values *Viz*. pH- 3.0, 5.0, 7.0, 9.0 and 11 using phosphate buffer. Cell density was adjusted to 10^6 -10^7 CFU/ml on the basis when culture reaches to 0.1 optical units at 600nm with spectrophotometer (Schaad, 1992). Seventy two hours old bacterial and fungal culture (10^6-10^7 CFU/ml) was serially diluted from 10^{-1} to 10^{-5} using 9ml sterile distilled water blanks. 100µl of each dilution was spreaded separately on to the sterile nutrient glucose agar plate having different pH range. All the inoculated plates were incubated at 30^{0}C temperature for 72 hours. After incubation, results were recorded for the development of colonies on the media having different pH. Number of colonies were counted and recorded (Suresh *et al*., 2013; Yenjerappa, 2009).

2.3 Effect of Salt (NaCl) Concentration on Growth of the Pathogen

The effect of sodium chloride (NaCl) as a salt on the growth of isolated promising cultures was studied by adjusting the sodium chloride concentration of the medium *Viz*. 0.05%, 0.25%, 0.5%, 1.0% and 1.5% of NaCl. Cell density was adjusted to 106 -107CFU/ml on the basis when culture reaches 0.1 optical units at 600nm with spectrophotometer (Schaad, 1992). Seventy two hours old bacterial fungal culture (10^6 – 10^7CFU/ml) was serially diluted from 10^{-1} to 10^{-5} by using 9 ml sterile distilled water blanks. 100µl of each dilution was spreaded separately on sterile nutrient glucose agar plate having varying salt concentrations as stated above. All the inoculated plates were incubated at 30^{0}C temperature for 72 hours. After incubation, results were recorded for the development of colonies on the media having different sodium chloride (NaCl) concentrations. Number of colonies were counted and recorded (Suresh *et al*., 2013; Yenjerappa, 2009).

III. RESULTS AND DISCUSSIONS

3.1 Effect of Different Temperatures on growth of the Bacterial Pathogens

The maximum colony number of the pathogens was observed at 30^{0}C which was followed by 25 ^{0}C, 35^{0}C. The minimum colony number of the pathogen was observed at 40^{0}C, 20^{0}C which was followed by 15^{0}C. No growth of the pathogens was observed at 10^{0}C and 50^{0}C on sterile nutrient glucose agar (**Table 1**).

Table 1: Effect of Different Temperatures on growth of the Bacterial pathogens of Pomegranate

Different Temperatures (^{0}C)	**Number of colonies x 10^5 CFU/ml**		
	Xanthomonas axonopodis VMB13	*Xanthomonas campestris VMB15*	*Xanthomonas vesicatoria VMB17*
10	00	00	00

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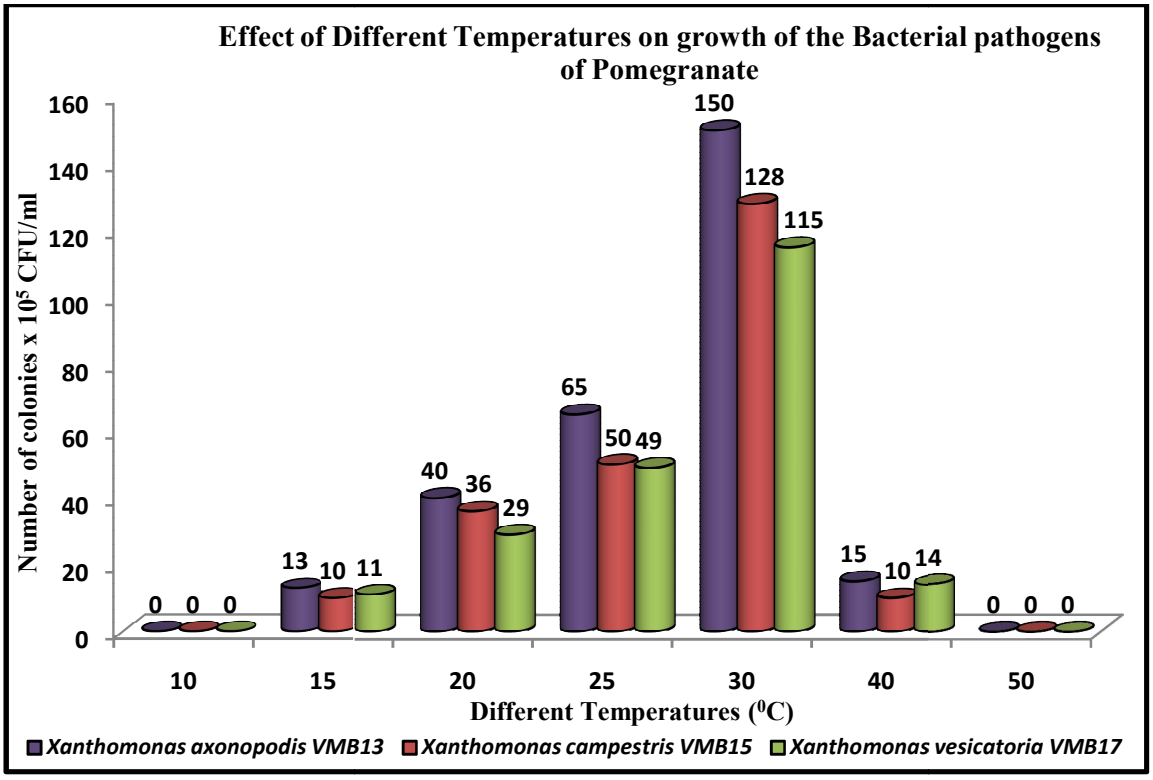
15	13	10	11
20	40	36	29
25	65	50	49
30	150	128	115
40	15	10	14
50	00	00	00

Figure 1: Effect of Different Temperatures on growth of the Bacterial pathogens of Pomegranate



The incubation temperature has a profound effect on the growth of bacterial pathogens. The temperature is one of the critical factors that have direct effect on growth of bacteria. Temperature affects the requirements of nutrient, the biomass composition and the nature of the metabolism in the cultures. The effects of different temperatures on the growth rate over most of its range can be predicted in terms of activation energy for growth. This relation breaks down near to both the upper temperature limits and lower temperature limits for growth. Above the optimum temperature, probably cell degradation becomes dominant over the growth process and near the lower temperature limit the regulation of metabolism may fail (Hang and Woodams, 1998).

The effect of different temperature on growth rate of different bacterial pathogens causing several diseases in Pomegranate was determined by culturing the bacterium individually in the production media and incubated at different temperatures. The experiments were carried out individually at different temperatures such as 10^0C, 15^0C, 20^0C, 25^0C, 30^0C, 40^0C and 50^0C. The measurement of number of colonies developed after incubation was carried out after incubation at different temperatures and the results were recorded.

It evident from the **Figure 1** that the number of colonies developed by the given pathogens *Viz. Xanthomonas axonopodis VMB13, Xanthomonas campestris VMB15* and *Xanthomonas vesicatoria VMB17* at different temperatures such as 10^0C, 15^0C, 20^0C, 25^0C, 30^0C, 40^0C and 50^0C was up to 00, 13, 40, 65, 150, 15 and 00 respectively by the isolate VMB13 whereas 00, 10, 36, 50, 128, 10 and 00 by the isolate VMB15 and 00, 11, 29, 49, 115, 14 and 00 by the isolate VMB17.

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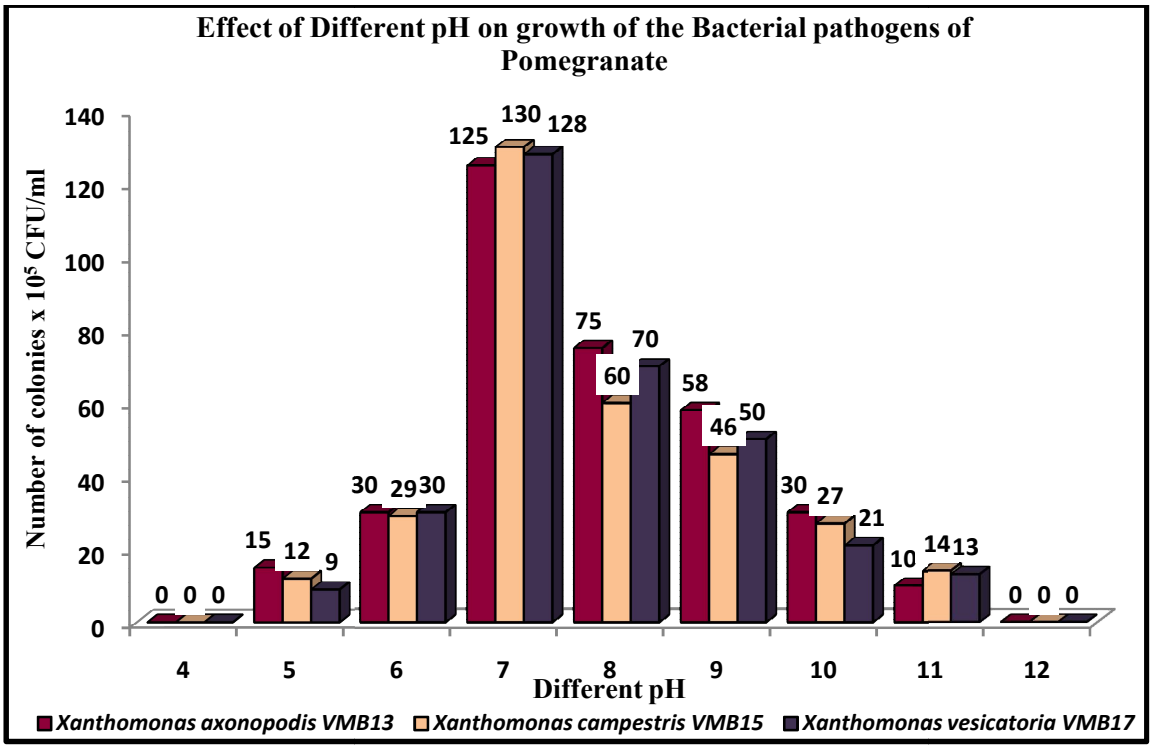
The effect of different temperatures on growth rate of the bacterial pathogens of Pomegranate was studied. A temperature of 30^0Cwas found optimal for maximum of all the three bacterial pathogens under study i.e. VMB13, VMB15 and VMB17. Further increase or decrease in temperature gradually decreased the growth of the pathogens. From the results depicted in **Figure 1 and Table 1** the optimum temperature for the growth of *Xanthomonas axonopodis VMB13, Xanthomonas campestris VMB15* and *Xanthomonas vesicatoria VMB17* is 30^0C.

Table 2: Effect of Different pH on growth of the Bacterial pathogens of Pomegranate

Different pH	Number of colonies x 10^5 CFU/ml		
	Xanthomonas axonopodis VMB13	*Xanthomonas campestris VMB15*	*Xanthomonas vesicatoria VMB17*
4.0	00	00	00
5.0	15	12	09
6.0	30	29	30
7.0	125	130	128
8.0	75	60	70
9.0	58	46	50
10	30	27	21
11	10	14	13
12	00	00	00

In the present study, the effect of varying initial pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 11 and 12) on growth of *Xanthomonas axonopodis VMB13, Xanthomonas campestris VMB15* and *Xanthomonas vesicatoria VMB17* was studied. Culture pH has a profound effect on the growth of all the bacterial pathogens in the study was investigated since, certain enzymes are pH sensitive. Therefore, maintenance of an appropriate initial pH for the growth of pathogens is vital. The initial pH of basal medium determines the optimal bacterial growth. Initial pH of the basal medium has a direct influence on bacterial metabolism.

Figure 2: Effect of Different pH on growth of the Bacterial pathogens of Pomegranate



It evident from the **Figure 2** that the number of colonies developed after incubation by the given pathogens *Viz. Xanthomonas axonopodis VMB13, Xanthomonas campestris VMB15* and *Xanthomonas vesicatoria VMB17* at different

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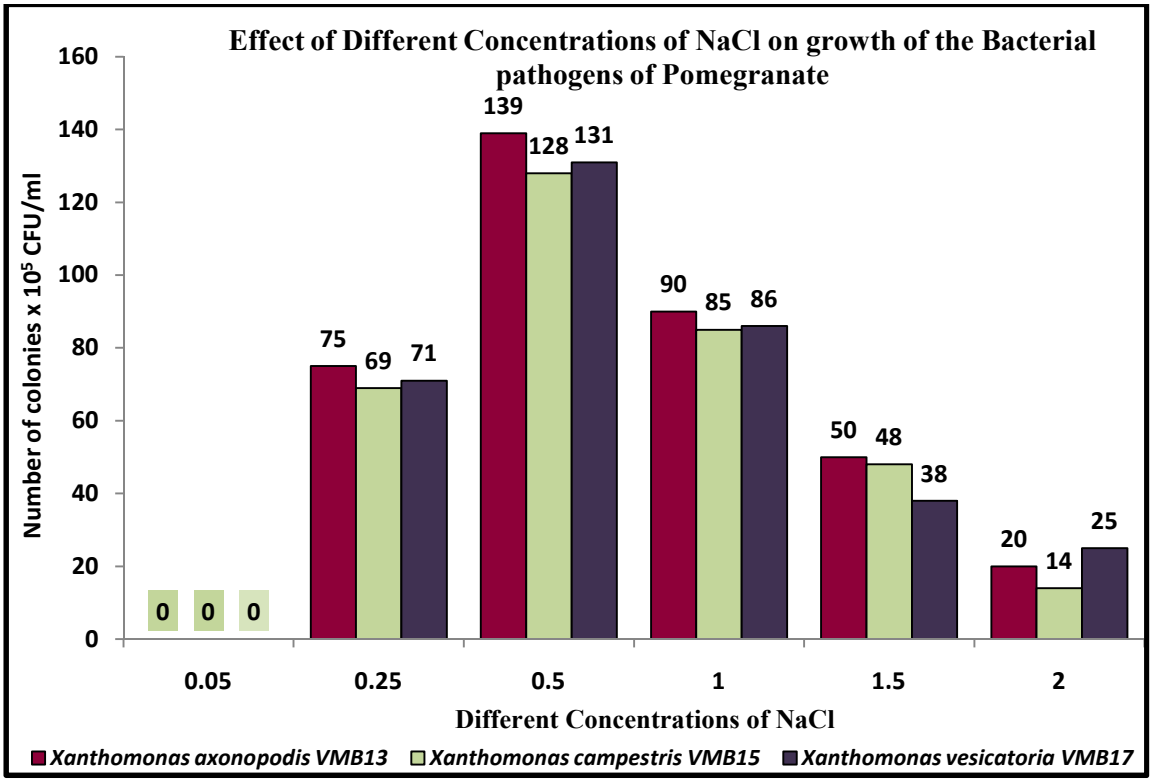
pH such as (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 11 and 12) was up to00, 15, 30, 125, 75, 58, 30, 10 and 00 whereas 00, 12, 29, 130, 60, 46, 27, 14 and 00 and 00, 09, 30, 128, 70, 50, 21, 13 and 00 respectively.

The effect of initial pH on the growth of the bacterial pathogens was investigated **(Figure 2)**. The result showed that an increase in pH brought about decrease in the number of colonies on the agar medium. The large number of development of colonies was obtained at pH 7.0. Further increase in pH was found to decrease the number of colonies on the agar medium. In this way we can say that the optimum pH for the growth of bacterial pathogens *Viz*. *Xanthomonas axonopodis VMB13, Xanthomonas campestris VMB15* and *Xanthomonas vesicatoria VMB17* of Pomegranate is pH 7.0.

Table 3: Effect of Salt (NaCl) Concentrations on growth of the Bacterial pathogens of Pomegranate

Salt Concentrations (%)	Number of colonies x 10^5 CFU/ml		
	Xanthomonas axonopodis VMB13	*Xanthomonas campestris VMB15*	*Xanthomonas vesicatoria VMB17*
0.05	00	00	00
0.25	75	69	71
0.5	139	128	131
1.00	90	85	86
1.50	50	48	38
2.00	20	14	25

Figure 3: Effect of Different Concentrations of NaCl on growth of the Bacterial pathogens of Pomegranate



The maximum number of colonies of the pathogen was observed on agar medium with 0.5% sodium chloride (NaCl) concentration followed by 0.25% and 1.0% salt concentration. Lowest colonies were observed on NG medium with 1.5%, 0.05 % sodium chloride concentration respectively.

Basamma (2013) reported that zero percent NaCl concentration was found to be most suitable for the growth of isolates (*Xanthomonas axonopodis pv. punicae*) followed by 1% concentration preferred by all isolates.

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