International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 1804-1806

Received: 10-07-2017
Accepted: 11-08-2017

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In vitro evaluation of synthetic antibiotics and chemicals against *Xanthomonas axonopodis* pv. *punicae*

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Abstract
Three different chemicals viz. Nano copper, Hydrogen peroxide and streptocycline were tested *in vitro* at 100, 300 and 500 ppm against of *X. axonopodis* pv. *punicae* by using inhibition zone technique. Nano Copper @500 ppm was found most effective for controlling *Xanthomonas axonopodis* pv. *punicae* by forming 26.06 per cent inhibition. Same chemical @300 and @100 ppm found second and third best effective chemical which showed 20.22 per cent and 18.86 per cent inhibition, respectively followed by Streptocycline 10.98 per cent @ 500 ppm and Hydrogen Peroxide 7.31 per cent @ 500 ppm. Amongst all the chemicals lowest per cent inhibition was observed in the treatment of Streptocycline @100 ppm.

Keywords: *Xanthomonas axonopodis* pv. *punicae*, Nano Copper, Streptocycline

Introduction
Pomegranate (*Punica granatum* L.) is a favourite table fruit in tropical and sub-tropical regions of the world which belongs to family *Punicae*. Since 2002, the pomegranate bacterial blight disease has reached the alarming stage and hampering the Indian economy and export of quality fruits. The disease accounted up to 70 – 100 per cent losses during 2006 in Karnataka, Maharashtra, Andhra Pradesh and Tamil Nadu resulting in abandoning many pomegranate orchards. During the year 2007, the total output of pomegranate production in India was down by 60 per cent (Raghavan, 2007) [6]. In the orchard health management, streptocycline, the most common antibiotic is being used in a dose (>500 ppm) more than twice the recommended dose (100-200 ppm). Keeping this in view, alternate source for management of bacterial blight needs to be studied. Therefor the possible mode of action of nanobased formulation and hydrogen peroxide against pomegranate bacterial blight disease is studied *in vitro*.

Material and Methods
Different chemicals at concentration 100, 300, 500 ppm were evaluated *in vitro* applying inhibition zone technique (paper disc method) and using Nutrient Agar (N.A.) as basal culture medium.

Fresh Nutrient Agar medium was prepared and dispersed in 100 ml quantities in conical flask (200 ml. Cap.), plugged and autoclaved at 15 lbs/cm^2 pressure for 15-20 minute. The desired concentration of chemicals i.e. 100, 300, 500 ppm was prepared by using appropriate quantities of chemical required for 100, 300, 500 ppm concentration. In this desire concentration of chemicals 10 mm discs of Whatman No. 1 filter paper was dipped for few minutes. After sterilization of media, it was allowed to cool down to 35°C before pouring. Approximately 20 ml liquid media was poured in previously sterilized petri plates and allowed them to solidify. Pouring of plates were always be done by using Laminar Air Flow cabinet under aseptic condition. After solidification of media of petri plates, the bacterial suspension was spread on Nutrient Agar with glass spreader. After uniform spreading of bacterial suspension 10 mm disc of Whatman No.1 filter paper previously dipped in desired concentration of chemicals was placed in centre of medium. Three replication for each concentration of chemical was maintained. Plates containing Nutrient Agar with bacterial suspension without any chemical was maintained as control. The experiment was conducted in completely randomized design with three replication and ten treatments as given below.

Treatment details:

T1	:	Nano copper @ 100 ppm
T2	:	Nano copper @ 300 ppm
T3	:	Nano copper @ 500 ppm
T4	:	Hydrogen peroxide @ 100 ppm
T5	:	Hydrogen peroxide @ 300 ppm
T6	:	Hydrogen peroxide @ 500 ppm
T7	:	Streptocycline @ 100 ppm
T8	:	Streptocycline @ 300 ppm
T9	:	Streptocycline @ 500 ppm
T10	:	Contol (untreated)

All these petri plates after treatment were incubated at 28 ± 2°C for 48 hours. Observation on radial growth of test pathogen and per cent inhibition over control was calculated by the formula of Ashoka *et al*. (1982).

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent inhibition,
C = Growth of test pathogen in control plate,
T = Growth of test pathogen in treatment plate

Results and Discussion

The result presented in Table 1 revealed that Nano Copper @500 ppm found most effective for controlling *Xanthomonas axonopodis* pv. *punicae* by forming 26.06 per cent inhibition. Same chemical @300 and @100 ppm found second and third best effective chemical which shows 20.22 per cent and 18.86 per cent inhibition respectively followed by Streptocycline 10.98 per cent @ 500 ppm and Hydrogen Peroxide 7.31 per cent @ 500 ppm. Amongst all the chemicals lowest per cent inhibition was observed in the treatment of Streptocycline @100 ppm.

The results obtained on control strategies correlates with the results of earlier workers Desai *et al.* (1967) and Raut *et al.*, (2010) [7]. Mondal and Mani (2012) [5] reported the efficacy of nanocopper in suppression of growth as well as in the water soaked lesions induced by *Xap*. The nanocopper suppressed *Xap* growth at 0.2 ppm, i.e., >10,000 times lower than that usually recommended for Copper oxychloride. Scanning electron microscopy (SEM) revealed cell wall degradation in nanocopper treated bacterial cells that failed to colonize plant tissue as well as to produce the characteristic intense water soaking. This study suggested that nanocopper could be employed as an environmentally friendly strategy for the management of pomegranate bacterial blight. Ambadkar *et al.*, (2015) [1] and Ashish *et al.*, (2016) [2] observed that Streptocycline was found effective at concentration >200 but less than 500 ppm.

Table 1: *In vitro* evaluation different chemicals against *Xanthomonas axonopodis* pv. *punicae.*

Treat. No.	Treatments	Mean colony growth (mm)*	% Inhibition
T1	Nano copper 100 ppm	73.01	18.86 (25.75)
T2	Nano copper 300 ppm	71.79	20.22 (26.72)
T3	Nano copper 500 ppm	66.54	26.06 (30.69)
T4	Hydrogen Peroxide 100 ppm	84.85	5.71 (13.82)
T5	Hydrogen Peroxide 300 ppm	83.88	6.79 (15.10)
T6	Hydrogen Peroxide 500 ppm	82.95	7.31 (15.68)
T7	Streptocycline 100 ppm	85.77	4.69 (12.50)
T8	Streptocycline 300 ppm	84.25	6.37 (14.61)
T9	Streptocycline 500 ppm	80.43	10.98 (19.35)
T10	Control	90.00	0.00
	S.E.±	0.39	1.17
	C.D.(P=0.01)	1.28	3.85

*Mean of three replications
Figures in parenthesis are arc sin values.

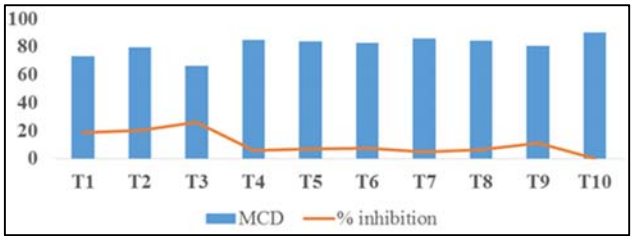

Fig 1: *In vitro* eva luation of different chemicals against *Xanthomonas axonopodis* pv. *punicae*

T_1- Nano Copper @ 100 ppm
T_2- Nano copper @ 300 ppm
T_3- Nano copper @ 500 ppm
T_4- Hydrogen peroxide @ 100 p
T_5- Hydrogen peroxide @ 300 ppm
T_6- Hydrogen peroxide @ 500ppm
T_7- Streptocycline @ 100 ppm
T_8- Streptocycline @ 300 ppm
T_9- Streptocycline @ 500 ppm
T_{10}- Control

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