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In vitro Efficacy of Different Chemicals, Botanicals and Bioagent Against *Xanthomonas axonopodis* pv. *punicae*

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ABSTRACT

Bacterial blight is the most severe disease of the Pomegranate, caused by bacterium Xanthomonas axonopodis pv. punicae. It is widely spread bacterial disease of plants from both epidemiological and economic points of view. The pathogen affects different plant parts like leaf, stem and fruit. Infection results in water soaked oily spot symptoms on leaves and fruits which consequently decreases fruit production and market value. Extensive agricultural practices along with large amount of pesticide as well as antibiotic are required to control this disease. Investigation was carried to study in vitro efficacy of different chemicals, botanicals and bioagent individually and in combination against Xanthomonas axonopodis pv. punicae by using disc diffusion method. Maximum zone of inhibition were recorded in chemical treatments. Copper oxychloride (0.3%) + Streptomycin sulphate (500 ppm) was found significantly superior in inhibiting the growth of bacteria with 14.33 mm zone of inhibition.

Key words: *Pomegranate, bacterial blight, Xanthomonas axonopodis, efficacy, disc diffusion method.*

INTRODUCTION

Pomegranate (*Punica granatum L.*) belongs to the family *Lythraceae*, is one of the favorite table fruit of tropical and subtropical regions. According to the data published by National Horticultural Board of India, the total area under cultivation of Pomegranate in India is 131.00 thousand ha and production is around 1346.00 thousand tons in 2013-14. Maharashtra is the leading producer of Pomegranate contributing about 70.2% of Pomegranate production followed by Karnataka, Gujarat, Andhra Pradesh, and Tamil Nadu. Bacterial blight is the most severe disease of the Pomegranate, caused by bacterium *Xanthomonas axonopodis pv. punicae* (Hingorani and Singh) Vauterin *et al*. Bacterial blight of Pomegranate was first reported in India from Delhi in 1952 [1].

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The disease at the initial stages cause small spots or chlortic lesions on the leaves later led to defoliation. On the fruits pathogen produces L or Y shaped cracks, cankerous lesions on stem and in severe cases plants dry leading to death of plants. Studies conducted in several parts of the world identified *Xanthomonas auxonopodis* pv. *punicae* as the causative organism of bacterial blight which is a gram negative short rod bacterium[2,3].

Management of bacterial blight of Pomegranate is a major concern. This disease could not be effectively managed with conventional antibiotics like Streptomycin in field conditions. Spraying streptomycin or copper oxychloride is the common mode of control of bacterial blight, however with limited success[4]. Recent survey conducted in Pune and Sangli district of Maharashtra has reported that farmers have tried different methods including spraying bleaching power, Bordeaux mixture, diaammonium phosphate, urea and farmyard manure to control the disease, but without any success[5].

Earlier studies showed that, bacterial blight disease more effectively inhibited by chemicals like Bordeaux mixture 1% [2,6] ,Streptocycline + Ampiclox (300ppm + 500ppm)[7], Cefoperozone[8], Streptocycline + copper oxychloride (0.05 % +0.25 %) and Streptocycline + copper hydroxide (0.05 % +0.25 %)[9]. Among botanicals, water extracts of *Mesua ferra* (commonly known as Nageswar)[10], Tulsi leaves extract[9], 10% Garlic bulb extract[2], Cow urine with *Helicteres isora Linn.* fruit extract were found effective in controlling of disease[11]. Different bioagents like *Bacillus subtilis, Pseudomonas fluorescens*[12]and *Trichoderma amatum*[8] were also shown good inhibitory activity against *X. xonopodis* pv. *punicae.*

Thus the investigations were carried out on management of disease with other chemicals individually and in combination.

MATERIALS AND METHODS

Collection of diseased sample and isolation of pathogen :

The bacterial blight diseased samples were collected from Ahmednagar district of Maharashtra state. The isolate of pathogen was obtained from infected leaves of Pomegranate showing typical symptoms of bacterial blight by tissue isolation method. A bacterial suspension of each specimen was then cultured on Nutrient Sucrose Agar (NSA) medium. Following incubation, colonies similar to *Xanthomonas* were maintained on NSA medium at room temperature by adopting subsequent sub culturing at periodical and regular intervals. Three days old cultures were used for further studies.

Pathogenicity test of the isolate :

For this study the healthy seedling of Pomegranate cultivar 'Bhagwa' was obtained from the central nursery of state agriculture university M.P.K.V. Rahuri, Dist. - Ahmednagar, Maharashtra state. The seedling was grown under aseptic *in vitro* condition and veinlets of some leaves were injected with 48 h old bacterial suspension with the help of sterile syringe (Plate 1). Some plant leaves were injured by sharp needle and inoculated by spraying bacterial suspension. The inoculated plants were covered with polythene sheets and incubated for 10-12 days at 25 to 28 ^{o}C temperature. After 10 – 12 days observations made for typical symptoms of bacterial blight on leaves, the organism was reisolated from artificially inoculated leaves and used for further antibacterial studies.

***In vitro* efficacy of different chemicals, botanicals and bioagent :**

Total 18 treatments were used to study the *in vitro* efficacy of the chemicals (T_1 to T_{11}), botanicals (T_{12} to T_{17}) and bioagent (T_{18}) against *Xanthomonas axonopodis* pv. *punicae* by using disc diffusion method (Table 1). The inhibitory activities of the chemicals individually and in combination were assessed by preparing the different concentrations by diluting the stock concentration depicted in table 1 (T_1 to T_{11}). Botanical extracts were prepared as, 100 grams of fresh sample was chopped and macerated in a surface sterilized mortar and pestle by adding 100 ml of sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth; filtrate thus obtained was used as a stock for antibacterial activity (T_{12} to T_{17}). Bioagent solution of *Bacillus subtilis* was prepared by

diluting the pure culture 8 times in water to get concentration $1x10^8$ (T_{18}). The bacterium *X. axonopodis* pv. *punicae* was multiplied by inoculating the loopful culture in 250 ml conical flask containing 100 ml of nutrient broth medium. The inoculated flasks were incubated at 28°C for 72 hours. The 20 ml bacterial suspension was added to molten cooled 1000 ml nutrient agar medium at temperature 45°C. The seeded medium was thoroughly mixed and poured into the sterilized petriplates and plates were allowed to solidify. The filter paper (Whatman No. 42) discs of 5 mm in diameter were prepared and soaked in the respective solution for five minutes and transferred onto the surface of the seeded medium in petriplates. The plates were incubated at 25 to 27 °C for 72 hours and the inhibition zone around the filter paper discs was measured. Paper disc soaked in sterile distilled water served as control (T_0).

RESULTS AND DISCUSSION

Isolation and pathogenicity test of bacterial culture :

Plates showing well separated, typical, yellow, mucoid, colonies of *Xanthomonas* bacterium were used to check pathogenicity on leaves of Pomegranate to confirm the isolate. The characteristic symptoms of the disease appeared after 10 to 12 days of inoculation in the form of 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 (Plate 2 a). Reisolation carried out from artificially inoculated plants yielded the bacterial colonies similar to the previous one (Plate 2 b).

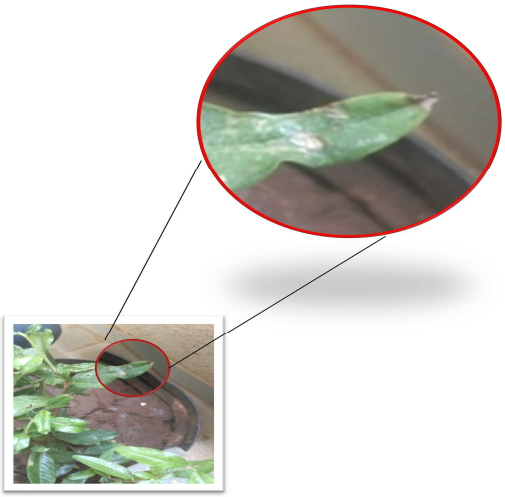
Efficacy of different chemicals, botanicals and bioagent against *Xanthomonas axonopodis* pv. *punicae* :

Various concentration and combination of chemicals, botanicals and bioagent were assessed *in vitro* against *Xanthomonas axonopodis* pv. *punicae*. The data presented below revealed the significant differences in zone of inhibition among the different treatments at different concentrations (Table 1).

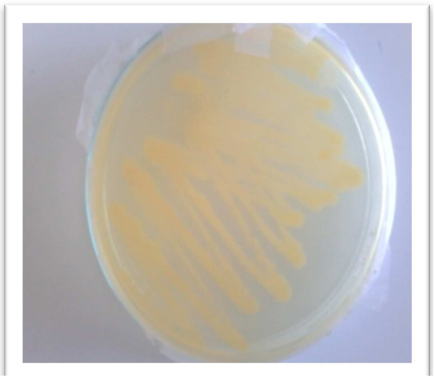
The treatment number T_{11} i.e. combination of COC (0.3%) + Streptomycin sulphate (500 ppm) was found significantly superior in inhibiting the growth of bacteria shown 14.33 mm zone of inhibition followed by treatment T_{10} i.e. COC (0.3%) + Streptomycin sulphate (400 ppm) shown 13.0 mm zone of inhibition (Plate 3 b). Among botanicals, Garlic bulb extract (10 %) showed maximum zone of inhibition i.e. 9.66 mm, followed by Parthenium leaves extract (10 %) 9.33 mm and Neem leaves extract (10 %) 8.33 mm (Plate 3 c) while *Bacillus subtilis* a bioagent shown 9.33 mm zone of inhibition (Plate 3 d).

The present findings are in corroborates with earliar studies in which Streptocycline + copper oxychloride (0.05 % +0.25 %) and Streptocycline + copper hydroxide (0.05 % +0.25 %) were found effective in minimizing disease incidence[9]. The maximum activity of streptocycline (250ppm) + copper hydroxide (0.3%); streptocycline (500ppm) + copper hydroxide (0.3%) had been reported[6]. 10% garlic bulb extract was significantly greater in efficacy for inhibiting the growth of the pathogen[2].

Plate 1 - Pathogenicity test of *X. axonopodis* pv. *punicae* on Pomegranate plant

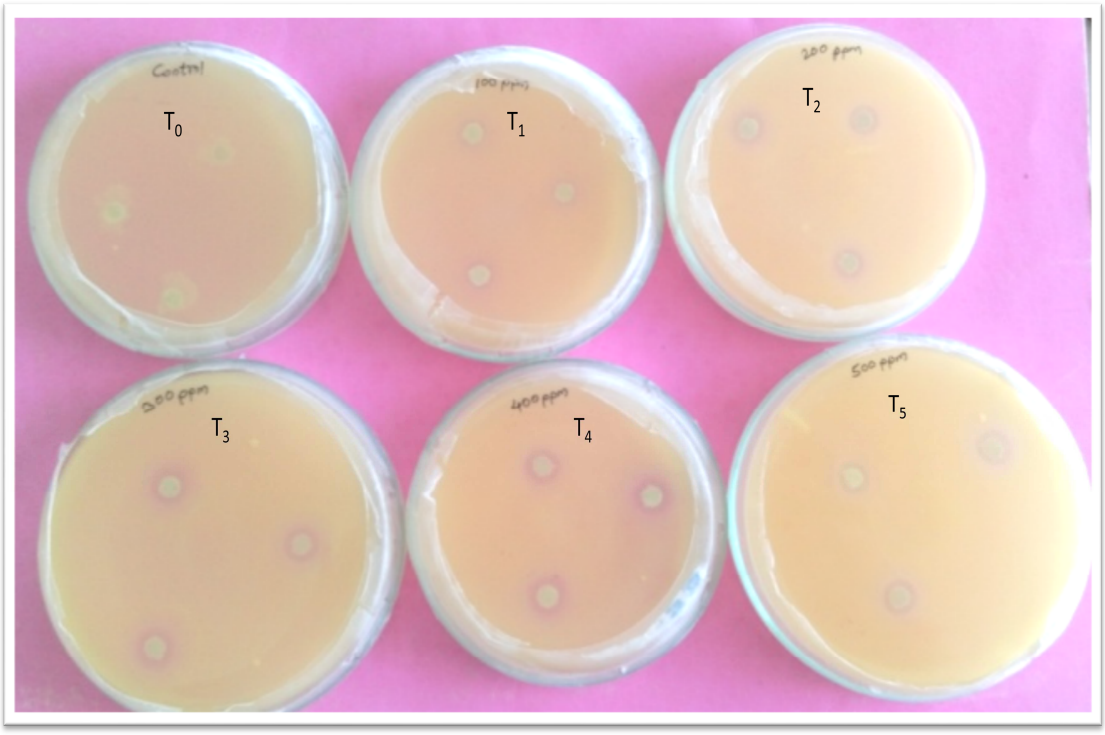


2. (a)

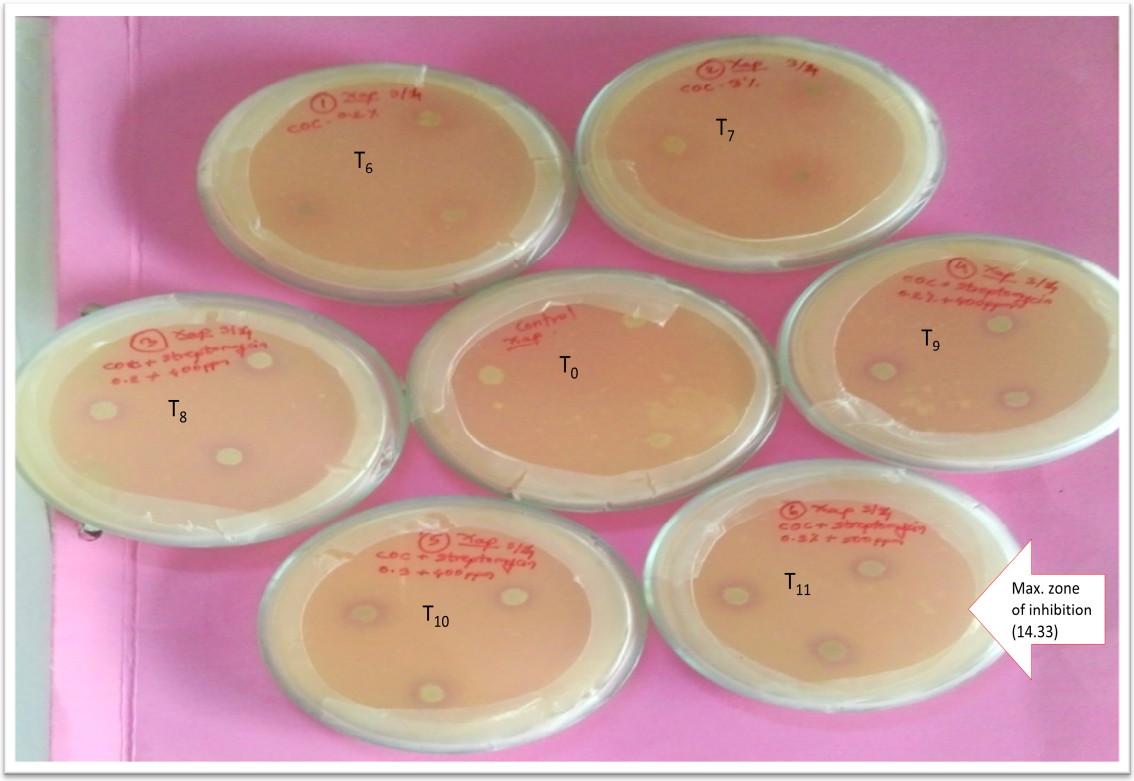


2. (b)

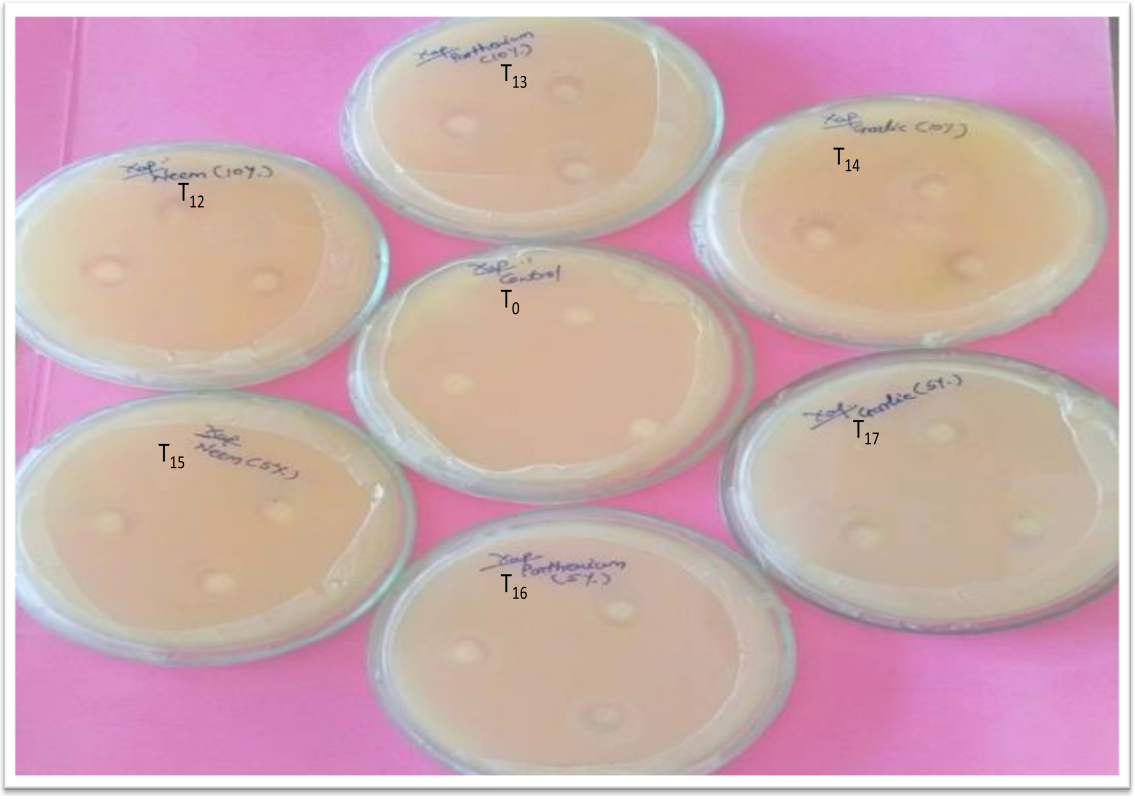
Plate 2- (a) Symptoms of bacterial blight observed on leaves after 10-12 days of inoculation. (b) Reisolated bacterial plate after pathogenicity test



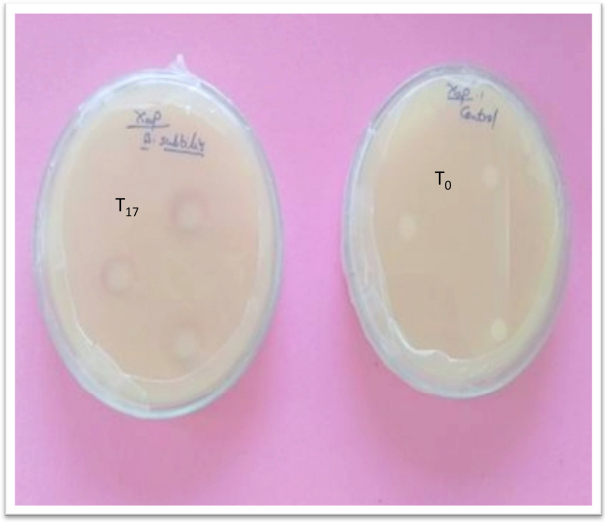
3. (a)


T6
T7
T0
T8
T9
T10
T11
Max. zone of inhibition (14.33)

3. (b)


T13
T12
T14
T0
T15
T17
T16

3. (c)



3. (d)

Plate- 3. Plates showing inhibitory activity of chemicals, botanicals and bioagent against *Xanthomonas axonopodis* pv. *punicae*

3. (a) Streptomycin sulphate

3. (b) COC and COC + Streptomycin sulphate

3. (c) Botanicals (Neem leaves, Parthenium leaves and Garlic bulb extracts)

3. (d) Bioagent – *Bacillus subtilis*

Table 1. *In vitro* efficacy of different chemicals, botanicals and bioagent against *Xanthomonas axonopodis* pv. *punicae* by disc diffusion method showing zone of inhibition (mm)

S. No.	Treatment	Concentration	Zone of inhibition (mm)
T_0	Control		00
T_1	Streptomycin sulphate	100 ppm	7.33
T_2	Streptomycin sulphate	200 ppm	8.00
T_3	Streptomycin sulphate	300 ppm	9.66
T_4	Streptomycin sulphate	400 ppm	10.0
T_5	Streptomycin sulphate	500 ppm	10.66
T_6	Copper oxychloride (COC)	0.2 %	10.33
T_7	Copper oxychloride (COC)	0.3 %	12.33
T_8	COC + Streptomycin sulphate	0.2 % + 400 ppm	10.66
T_9	COC + Streptomycin sulphate	0.2 % + 500 ppm	11.33
T_{10}	COC + Streptomycin sulphate	0.3% + 400 ppm	13.0
T_{11}	COC + Streptomycin sulphate	0.3% + 500 ppm	14.33
T_{12}	Neem leaves extract	5 %	6.33
T_{13}	Neem leaves extract	10 %	8.33
T_{14}	Parthenium leaves extract	5 %	6.66
T_{15}	Parthenium leaves extract	10 %	9.33
T_{16}	Garlic bulb extract	5 %	7.33
T_{17}	Garlic bulb extract	10 %	9.66
T_{18}	*Bacillus subtilis*	$1x10^8$	9.33

CONCLUSION

On the basis of the present *in vitro* study, it could be concluded that the maximum zones of inhibition were recorded in chemical treatments as compare to botanicals and bioagent. Copper oxychloride (0.3%) + Streptomycin sulphate (500 ppm) were found significantly superior in inhibiting the growth of bacteria with 14.33 mm zone of inhibition.

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