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Research Article

IN VITRO EFFICACY OF DIFFERENT CHEMICALS AND BIOLOGICAL AGENTS AGAINST *XANTHOMONAS CAMPESTRIS* PV. *VITICOLA* CAUSING BACTERIAL LEAF SPOT OF GRAPES

KAMBLE AMIT K.[1,2], SAWANT SANJAY D.[1], SAHA SUJOY[1]* AND SAWANT INDU S.[1]

[1]ICAR - National Research Centre for Grapes, Pune, 412307, Maharashtra, India
[2]Department of Agrochemicals and Pest Management, Shivaji University, Kolhapur, 416 004, Maharashtra, India
*Corresponding Author: Email-sujoyta@gmail.com

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Abstract- Bacterial leaf spot caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye has emerged as an important disease in grape growing regions of Maharashtra. In this study the bactericidal effect of different chemicals and biological agents were assessed in *in-vitro* conditions. Eleven different commercially formulated chemicals (streptocycline, bronopol, mancozeb, copper sulphate, copper oxychloride, copper hydroxide, carbendazim, difenoconazole, validamycin, kasugamycin and potassium phosphite) were tested at 50, 100, 500, 1000, 2000 and 3000 ppm concentrations. Three biological agents *viz.*, *Bacillus subtilis*, *Trichoderma as perolloides* and *Pseudomonas fluorescens* were also evaluated against *X. campestris* pv. *viticola*. Among different chemicals, streptocycline, mancozeb and bronopol showed significant inhibition of the pathogen while kasugamycin, copper oxychloride and copper hydroxide exhibited a low efficacy with respect to control of the bacteria. Copper sulphate, validamycin, difenoconazole, carbendazim and potassium phosphate showed no inhibition at all tested concentrations. In biological agents *B. subtilis* and *T. asperolloides* have potential against *X. campestris* pv. *viticola*, pathogen of bacterial leaf spot disease of grapes.

Keywords- Bacterial leaf spot, Bio-efficacy, Biological agents, Different chemicals, *in vitro, X. campestris* pv. *viticola*

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Introduction

Grape (*Vitis vinifera* L.) is an important fruit crop of India having both commercial and nutritional value. It is consumed as fresh or as processed food like wine and raisins. Grape cultivation is the most beneficial farming enterprises in recent years because of its phenomenal yield potential and contribution to generate employment opportunities. In India, Maharashtra is the leading state in production with an area of 90,000 ha under viticulture followed by Karnataka, Andhra Pradesh and Tamil-Nadu. During the year 2015-16, India has exported 1,56,218.34MT of grapes worth Rs. 1,551.32 crores [3].

Grape has been affected by several diseases and among them bacterial leaf spot (BLS) disease caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye (1972) is of considerable importance. The symptoms of BLS disease of grapes are characterized by water soaked lesions on leaves which in later stages become dark brown to black colour. The necrotic-cankerous lesions also develop on shoots and petioles and under favorable condition it causes up to 60-80% losses [6]. Over the years most of the farmers preferred copper based fungicides for management of BLS disease but now they have been reported to be resistant to pathogen [7]. As the present commercial cultivars of grapevines are highly susceptible to BLS disease [10], there is a need to search for new chemicals and biological agents for the control of BLS disease of grapes.

Therefore, the aim of this investigation was to evaluate *in vitro* various chemicals and biological agents for their efficacy and to find out the promising alternative option for controlling the *Xanthomonas campestris* pv. *viticola*.

Materials and Methods

In vitro efficacy of different chemicals

Eleven commonly used chemicals *viz.,* streptocycline (streptomycin sulphate 90% + tetracyline hydrochloride 10% w/w), bronopol (2-bromo-2-nitro-propane- 1,3 diol 95% w/w), mancozeb 75% WP, copper sulphate 99%, copper oxychloride 50% WP, copper hydroxide 77% WP, carbendazim 50% WP, difenoconazole 25% EC, validamycin 3% SL, kasugamycin 3% SL and potassium phosphite 95% were evaluated under *in vitro* conditions against *X. campestris* pv. *viticola.*

Stock solutions of 10,000 parts per million (ppm) of each chemicals were prepared by sterile distilled water (SDW). Further, different concentrations *viz.,* 50, 100, 500, 1000, 2000 and 3000 ppm were made by using the stock solution. *In vitro* efficacy of different chemicals was done by disc diffusion method [5] with certain modifications. Five replicates were maintained for each concentration.

The inoculum was prepared by pouring 10 ml of SDW over a 24 hr old culture plate and bacterial growth was gently scraped with the help of sterile nichrome wire loop and collected in a sterile tube. The bacterial suspension was adjusted to a density of 10^8 cfu/ml with the help of SDW by using McFarland's standards [10]. A sterile cotton swab was dipped into the bacterial suspension and bacterial lawn was made on nutrient agar (HiMedia M001). The inoculated plates were kept aside for drying the lawn. The sterilized filter paper (Whatman filter paper no. 1) discs (6 mm in diameter) were soaked in the solutions of different concentrations of above mentioned chemicals for 10 minutes. The chemical impregnated discs were placed onto the lawn with the help of sterilized forceps. The inoculated plates were incubated in the refrigerator at 5°C for four hours to allow the diffusion of chemical into the medium. Then the plates were incubated at 28 ±1°C for 72 hr. Lysis of the bacterial lawn around the disc was recorded and the results of sensitivity were reported as the zone of inhibition. The sterilized paper discs impregnated with SDW served as control.

In vitro efficacy of different biological agents

Cultures of *Bacillus subtilis*, *Trichoderma asperolloides* and *Pseudomonas fluorescens* were obtained from the plant pathology laboratory of ICAR-NRCG, Pune and used as biocontrol agents. The assay for antagonism was performed with certain modifications by the dual culture method [2] and cross streak method [14]. Six replications for each treatment were maintained.

The 5 mm of *T. asperolloides* mycelia agar disc was cut from a 72 hr old culture and placed at the center of Petri plates containing half-strength of each nutrient agar and potato dextrose agar media [16]. At the same time loopful of *X. campestris* pv. *viticola* was streaked at the two sides 3 cm away from *T. asperolloides* disc. The inoculated plates were incubated at 28±1°C for 8-10 days and observed for growth and spores formation of *T. asperolloides.* Separately inoculated *X. campestris* pv. *viticola* and *T. asperolloides* plates served as controls.

B. subtilis and *P.fluorescens* were streaked in a single line at the center of the nutrient agar plates and incubated at 28 ±1°C for 24 hr. After 24 hr *X. campestris* pv. *viticola* was streaked perpendicularly while maintaining 3 mm distance from central streak. The inoculated plates were incubated at 28±1°C for 24-48 hr and observed for lysis of the pathogen and length of inhibition was measured.

Statistical analysis

Recorded data were analyzed through Analysis of Variance (ANOVA) and treatments means were compared by Fisher's Least Significant Difference (LSD) test. Data was processed statistically through SAS (9.3) software.

Results

In vitro efficacy of different chemicals

Efficacy of different chemicals at different concentrations were evaluated by measuring the diameter of the inhibition zone against the *X. campestris* pv. *viticola*. The results are presented in [Table-1] and [Fig-1]. Inhibition of 11.6 mm and 9.4 mm at the lowest concentration of 50 ppm was exhibited by mancozeb 75% WP [Fig-2-b] and streptocycline 90% w/w [Fig-2-a] respectively. At highest concentration of 3000 ppm they showed inhibition zone of 21.6 mm and 19.8 mm respectively. Bronopol did not show any inhibition at 50 and 100 ppm concentration but at 500 ppm it had a zone of inhibition of 14.2 mm [Fig-2-c]. At maximum concentration of 3000 ppm bronopol showed 19.6 mm zone of inhibition. The chemicals *viz.,* copper oxychloride 50% WP, copper hydroxide 77% WP, did not inhibit *X. campestris* pv. *viticola* up to 1000 ppm concentration but at 2000ppm they showed 2.6mm and 5.6 mm inhibition zone. At highest concentration of 3000 ppm, inhibition zone was showed by copper oxychloride (7.8 mm) and copper hydroxide (7.8 mm) respectively [Fig-2-d and 2-e]. Kasugamycin 3% SL did not show any inhibition up to 2000 ppm but at 3000 ppm it showed 8.4 mm zone of inhibition. The remaining chemicals *viz.,* validamycin 3% SL, copper sulphate 99%, difenoconazole 25% EC, carbendazim 50% WP and potassium phosphite 95% did not show any inhibition for all the tested concentrations.

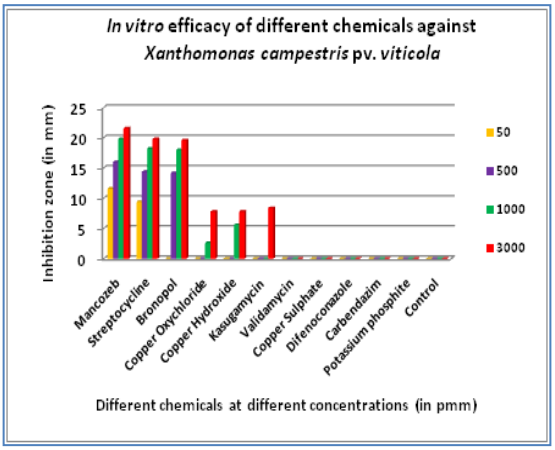


Fig-1 _In vitro_ efficacy of different chemicals against _X. campestris_ pv. _viticola_.

In vitro efficacy of different biological agents

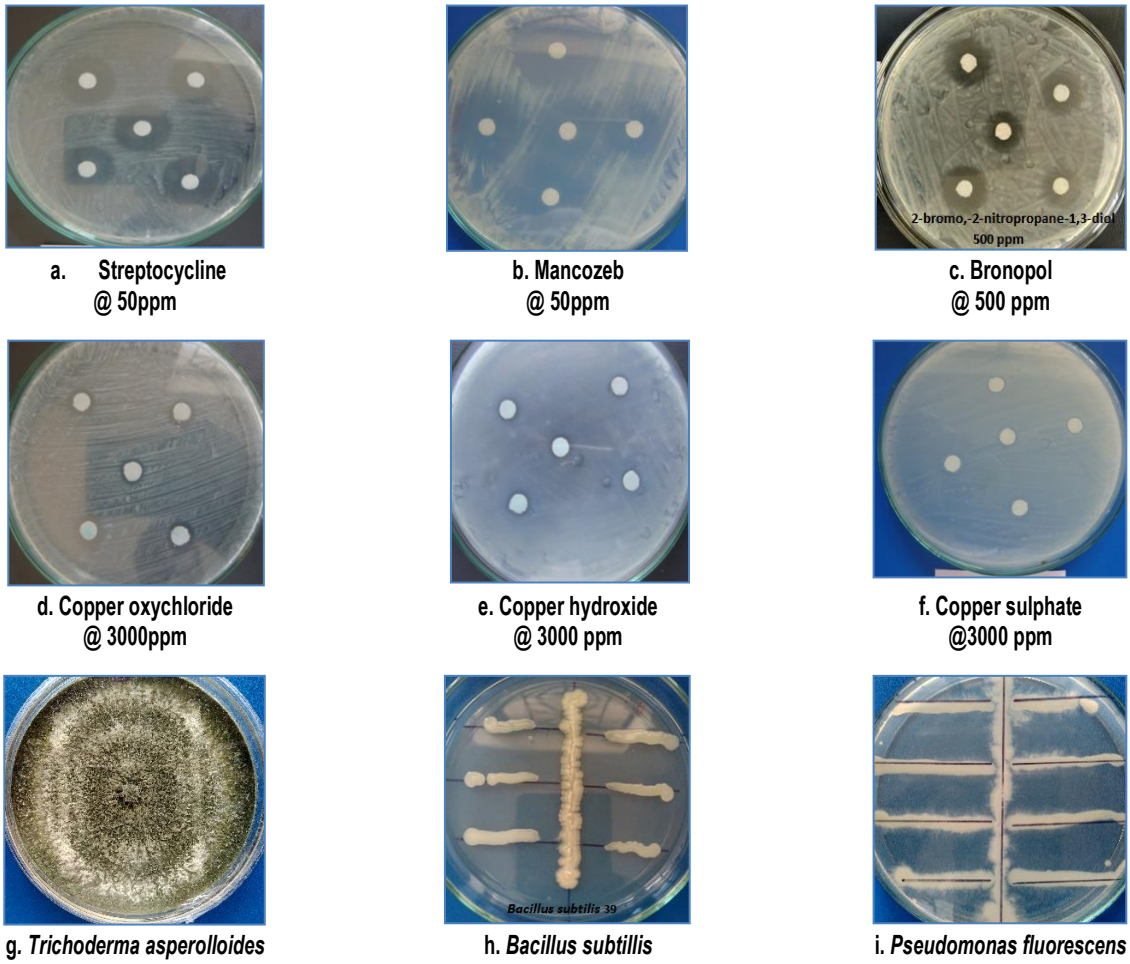
B. subtilis and T. asperolloides significantly inhibited the growth of *X. campestris* pv. *viticola*. The length of inhibition of *X. campestris* pv. *viticola* exhibited by *B. subtilis* 8.33 mm after 24 hr incubation at 28±1°C [Fig-2-g]. *T. asperolloides* completely over grew *X. campestris* pv. *viticola* and after 10 days of incubation it produced spores [Fig-2-h]. *P. fluorescens* did not showed any inhibitory effect against *X. campestris* pv. *viticola* [Fig-2-i].

Table-1 In vitro efficacy of different chemicals against *X. campestris* pv. viticola

Sr. no	Chemicals	Concentrations (in ppm)					
		50	100	500	1000	2000	3000
		Inhibition zone (in mm)					
1	Mancozeb	11.6a	13.6a	16a	18a	19.8a	21.6a
2	Streptocycline	9.4b	10.2b	14.4b	16.6b	18.2a	19.8b
3	Bronopol	0.00c	0.00c	14.2b	16.2b	18a	19.6b
4	Copper Oxychloride	0.00c	0.00c	0.00c	0.00c	2.6c	7.8d
5	Copper Hydroxide	0.00c	0.00c	0.00c	0.00c	5.6b	7.8d
6	Kasugamycin	0.00c	0.00c	0.00c	0.00c	0.00d	8.4c
7	Validamycin	0.00c	0.00c	0.00c	0.00c	0.00d	0.00e
8	Copper Sulphate	0.00c	0.00c	0.00c	0.00c	0.00d	0.00e
9	Difenoconazole	0.00c	0.00c	0.00c	0.00c	0.00d	0.00e
10	Carbendazim	0.00c	0.00c	0.00c	0.00c	0.00d	0.00e
11	Potassium phosphite	0.00c	0.00c	0.00c	0.00c	0.00d	0.00e
12	Control	0.00c	0.00c	0.00c	0.00c	0.00d	0.00e
LSD (0.05)		**0.47**	**0.22**	**0.64**	**0.42**	**3.81**	**0.58**

Discussion

Mancozeb 75% WP and streptocycline 90% w/w revealed best *in vitro* control which is analogous to the previous findings [17] wherein it was reported that *in vitro* streptocycline followed by mancozeb was highly effective against *Xanthomonas campestris* pv. *campestris* causing black rot of cabbage. All strains of bacterial pathogens of fruit plants *viz., Erwinia amylovora*, *X. arboricola* pv. *corylina*, *X. arboricola* pv. *juglandis*, *Pseudomonas syringae* pv. *syringae* and *Agrobacterium tumefaciens* were inhibited by mancozeb [12]. Streptomycin was found most effective against bacterial leaf spot of betelvine caused by *X. campestris* pv. *betlicola* [18]. Bronopol showed significant zone of inhibition at 500 ppm which corroborate with the previous results [19] which stated that bronopol have antibacterial activity against *X. axonopodis* pv. *punicae* causing bacterial blight of pomegranate. Jambenal et. al., [9] reported the efficacy of copper based fungicides in the control of *X. campestris* pv. *viticola*, which was in contradiction to the present findings. Both copper oxychloride 50% WP and copper hydroxide 77% WP exhibited very low level of inhibition at higher concentration of 3000 ppm while copper sulphate did not show any inhibition at all concentrations. The low efficacy of copper compounds in the control of *X. campestris* pv. *vesicatoria* causing



a. Streptocycline @ 50ppm | b. Mancozeb @ 50ppm | c. Bronopol @ 500 ppm

d. Copper oxychloride @ 3000ppm | e. Copper hydroxide @ 3000 ppm | f. Copper sulphate @3000 ppm

g. *Trichoderma asperolloides* | h. *Bacillus subtillis* | i. *Pseudomonas fluorescens*

Fig-2 *In vitro* efficacy of chemicals and biological agents against *X. campestris* pv. *viticola*.

bacterial spot of sweet pepper was also reported [1]. The present findings support previous findings [6] where copper based fungicides were found resistant against *X. campestris* pv. *viticola.* The Brazilian strains of *X. campestris* pv. *Viticola* were more tolerant than the type-strain, collected in 1972 in India [11]. The reason for such tolerance needs to be investigated at the molecular level of the pathogen.

Present findings also resemble the earlier findings [13] regarding bio-control agents where *B. subtilis* showed good inhibition activity against *X. campestris* pv. *campestris* causing black rot of crucifers. *T. viride* have significant inhibitory effects against *X. axonopodis* pv. *punicae* [15] while *T. harzianum* exhibited maximum inhibition against *X. oryzae* pv. *oryzae* causing bacterial leaf blight of rice [7]. Antagonistic microbial agents can inhibitor suppress the growth of plant pathogens by producing antibiotics or producing larger populations by means of competition, and thus occupying and competing for the same ecological niche [4].

Conclusion

The present findings clearly hint that the chemicals *viz.,* streptocycline, mancozeb, bronopol and biological agents *viz., B. subtilis* and *T. asperolloides* have a great potential against BLS disease of grapes. In field multi locational trials needs to be conducted to ascertain the present findings.

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Abbreviations

ppm : Parts per million
viz., : Videlicet
pv. :Pathovar
ha : Hectare
MT : Metric Ton
Rs. : Rupees
% : Percentage
w/w : Weight by weight
WP : Wettable Powder
EC : Emulsifiable Concentrate
SL : Soluble (liquid) Concentrate
hr : Hour
cfu : Colony forming unit
ml : MiliLitres
no. : Number
mm : Mili Meter
°C : Degree centigrade
SAS : Statistical Analysis System
Fig. : Figure
LSD : Least Significant Difference
@ : At the rate of

Ethical approval:
This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: None declared

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