Research Paper :

Basic studies on *Xanthomonas campestris*.pv. *viticola* causing bacterial leaf spot of grape and evaluated *in-vitro* efficacy of different chemicals and bioagents against its growth

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SUMMARY

The bacterium isolated from infected leaves showing typical symptoms of bacterial spot, yielded, yellow mucoid, shiny, slimy, convex odorless round colonies on nutrient agar medium. Based on physiological, biochemical and morphological characteristics, the bacterium was identified as *Xanthomonas campestris* pv. *vitcola*, Among the different chemicals and bio-agents were tested under *in vitro,* the streptocyline 500 ppm plus copper oxyhloride 2000 ppm produced maximum inhibition zone (24,97 mm) followed by streptocycline 500 ppm (22.40 mm). Among the bioagents tested, the *Bacillus subtilis* 500 ppm recorded highest inhibition of radial growth (8.15 mm) than *pseudomonas fluorescens* 5000 ppm.(7.05 mm)

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Key words : *Xanthomonas campestris* pv. *viticola*, *Bacillus subtilis*, Chemicals, *In vitro*

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Grape (*Vitis vinifera* L.) is an important temperate fruit of the world, native of Europe and cultivating in both tropical and subtropical regions of the world. It is one of the important horticultural crop grown in India. Its contain vitamin 'A' and good source of biflorohoids known to be usefully in condition as pulpusa, capillary edema, radiation damage etc.

Maharashtra has the largest area of 41,400 ha. followed by Kanrataka. 9721 ha, total production 1, 67,044 tones with a productivity of 17.20 tones per ha (Vasantha Kumar, 2007). In Karnataka grapevine cultivation it spread across the Bijapur, Bagalkot, Raichuru, Koppala, Belgaum, Kolar, Bangalore districts (Anonymous, 2006).

Presently, the bacterial leaf spot of grapevine has became a regular problem in the major grape growing area of Northern Karnataka, especially in September pruned vineyards. This diseases was noticed for the first time at Tirupati (Andhra Pradesh) on *Vitis vinifera* cv. ANCB-E-SHCHI, during 1960 (Nyudu,1972) during 1984 this disease was appeared in epiphytotic form in the areas of Sangali and Solapur districts of Maharashtra, on cv. Thompson seedless (Patil, 1988). Yield loss was estimated approximately 60 to 70 per cent (Chand and Kishun,1900). The present study was under taken to know the isolated organism is *Xanthomonas campestris* pv. *viticola* or not with the help of morphological, physiological and biochemical tests, and also evaluated the efficacy of different chemicals and bio-agents against growth of *Xanthomonas campestris* pv. *viticola* under *in vitro* condition.

MATERIALS AND METHODS

Basic studies includes, isolation and identification of the pathogen, with the help morphological, and bio-chemical tests. The infected grape leaves showing typical symptoms of bacterial spots as minute water-soaked lesions on leaves especially on veins and veinlets, irregular to angular and cankerous were collected from major grape growing areas

of Northern Karnataka. The bacterium was isolated by extracting ooze in the sterile distilled water taken in test tubes followed by dilution plate technique on Nutrient agar medium. Small pieces of infected leaves were cut aseptically from the edge of typical spots along with a little position of healthy tissue and surface sterilized with 70 per cent alcohol or one per cent sodium hypochlorite solution, washed in three series of sterile distilled water to remove traces of alcohol, then leaf bits were suspended in a small test tube containing 3 ml of sterilized distilled water kept for ten minutes. When the water became slightly turbid due to oozing of bacterial cells from the cut ends of the diseased tissues, the bacterial suspension was serially diluted in 9 ml sterile distilled water. The one ml of diluted bacterial cell suspension was poured in to sterilized Petriplates containing Nutrient agar. The plates were rotated gently in clockwise and anticlockwise direction to distribute the bacterial cell suspension uniformly in plates and to obtain well separated bacterial colonies. The inoculated plates were incubated at 28^0C for 72 hours. Observation was made for development of well separated light yellow, convex, small bacterial colonies on nutrient agar medium.

Based on morphological, physiological and biochemical characters, the test bacterium was identified as *Xanthomonas campestris* pv. *viticola*. The morphological characteristics such as cell shape, gram reaction, capsule and spore staining characters of the isolate was studied as described by Society of American Bacteriologists, Bradbury (1970) and Schaad and Stall (1988). The physiological and bio-chemical characters of the bacterium was studied for hydrolysis of starch, gelatin liquefaction, indole production, hydrogen sulphide production. Urease production, cosine hydrolysis, catalase reaction, utilization of Aspariagine as sole source of carbon and nitrogen, methyl red reaction, reduction of nitrate to nitrate, oxidese reaction and acid production from different sugars *viz*., glucose, fructose, sucrose. The tests were conducted as per the methods described by Bradbury (1970) and Schaad and Stall (1988).

The various chemicals and bioagents shown in Table 2 were tested at different concentrations against growth of *Xanthomonas campestris* pv. *viticola* by paper disc method. The bacterium was multiplied by inoculating the cultures in Nutrient broth taken in Erleyenmayer's flask and incubated at 28^0C for 48 hours, then bacterial culture in broth was seeded to lukewarm Nutrient agar, the seeded medium was poured in to sterilized Petriplates and allowed to solidify. The chemicals solutions were prepared at different concentrations as given.

RESULTS AND DISCUSSION

The isolated organism from infected grape leaves showing typical symptoms (Fig. 1) of bacterial spot yielded, yellow mucoid, slimy, glistening, convex and round colonies (Fig. 2) on Nutrient agar medium. The similar results were obtained by Hayward (1983), Mariano and Gama (2005) and Viana (2006). The results of the various morphological, physiological and bio-chemical tests are given in Table 1. The bacterial cells were small rod shaped with rounded ends, monotrichously flagellated obligately aerobic, gram negative and 0. to 1.2 x 2.3 µm in size , on Sx agar produced a clear starch digestion zone (3-4mm). Nonspore forming, non-acid fast, and oxidase negative but not utilized asparagine as a sole source of carbon and nitrogen. It was positive for catalase reaction, liquefaction of gelatin and produced hydrogen sulphide gas and utilized glucose, fructose, sucrose for acid production (Fig. 3). The strain did not produced indole, it failed to reduce nitrate to nitrite but hydrolyzed the casein and negative for methyl red reaction and urease reaction. Similar results were enumerated by Bradbury (1970) and Schaad andn Stall (1988).

Table 1 : Morphological,physiological and biochemical characteristics of *Xanthomonas campestris* pv.*viticola* causing bacterial leaf spot of grapevine

Characters	Isolate
1. Morphology : shape	Small rods
a. Occurrence	In single
b. Flagelation	Monotrichous
Staining	
a. Gram reaction	G –ve
b. Capsule staining	+
c. Spore staining	-
d. Acid fast	-
Biochemical characters	
a. Utilization of glucose, sucrose, fructose for acid production	+
b. Utilization of asparagine as sole source of carbon and nitrogen	-
C. Catalase reaction	+
d. Gelatin liquefaction	+
e. Casein hydrolysis	+
f. Methyl red reaction	-
g. Reduction of nitrate to nitrite	-
h. Urease reaction	-
i. Indole production	-
j. Hydrogen sulphide production	+
k. Oxidase reaction	-
l. Starch hydrolysis	+

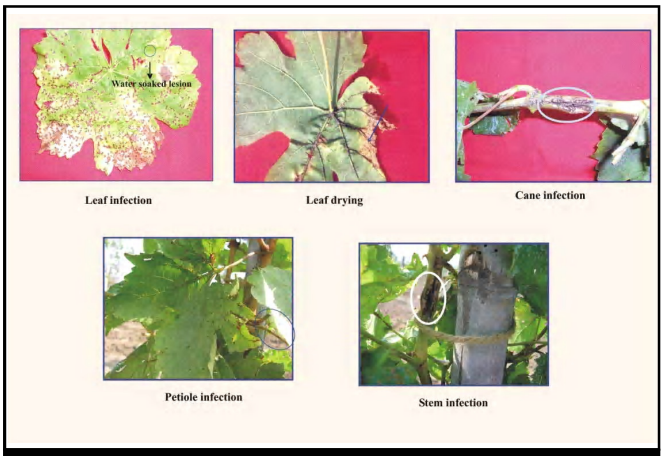

Fig. 1 : Symptomatology of bacterial leaf spot of grapevine

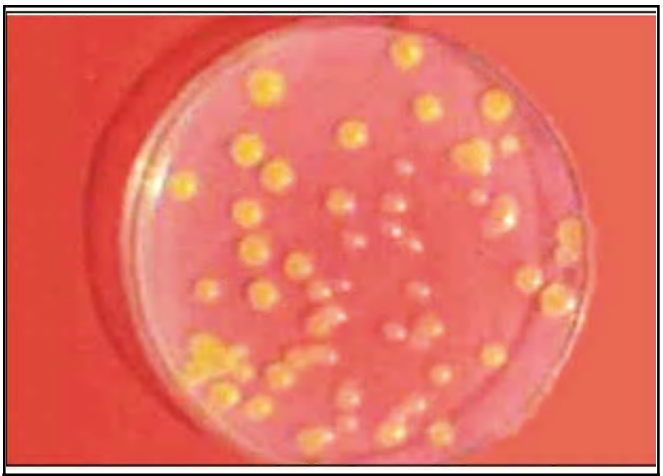

Fig. 2 : *Xanthomonas campestris* pv. *viticola* colonies on nutrient agar medium

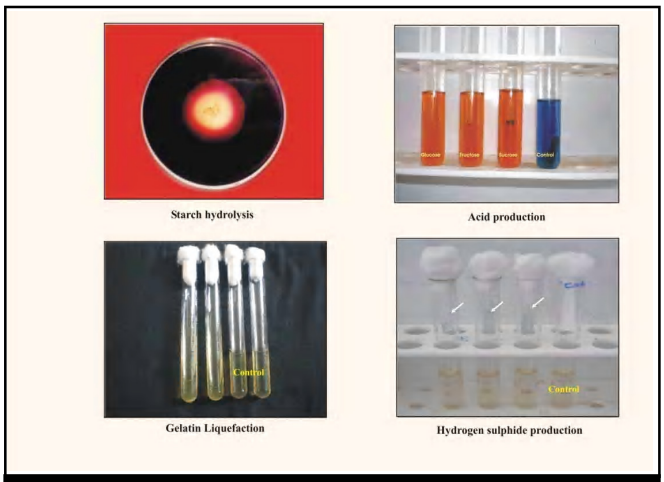

Fig. 3 : Biochemical tests for identification of causal organism

The efficiency of various chemicals and biogents were tested against growth of *Xanthomonas campestris* pv. *viticola*, causing bacterial leaf spot grapevine and the results expressed as inhibition zone (mm) are presented in Table 2 and Fig. 4 and 5. Its revealed that, Strptocycline 500 ppm plus Copper oxychloride 2000 ppm produced a highest inhibition zone of 24.97 mm followed by Strpcycline alone at 500 ppm has produced (22.40 mm) inhibition zone and it was on for with Agrimcyin 500 ppm plus Copper oxychloride 2000 ppm (21.70 mm),

Table 2 : *In-vitro* efficacy of different chemicals and bioagents against growth of *Xanthomonas campestris* pv. *viticola*

Sr. No.	Name of the chemicals and bioagents	Concentration (ppm)	Inhibition zone (mm)
1.	Streptocycline	500	22.40
2.	Bromopal	500	15.80
3.	Bacteriocare	500	12.20
4.	Bactrinol-100	500	16.90
5.	Agrimycin	500	19.54
6.	Streptocycline + Copper oxychloride	500 + 2000	24.97
7.	Bromopal + Copper oxychloride	500 + 2000	19.65
8.	Bacteriocare + Copper oxychloride	500 + 2000	21.40
9.	Bactrinol-100 + Copper oxychloride	500 + 2000	20.90
10.	Agrimycin + copper oxychloride	500 + 2000	21.70
11.	Copper oxychloride	3000	10.35
12.	*Pseudomonas fluorescens*	5000	7.05
13.	*Bacillus subtilis*	5000	8.15
14.	Control		0.00
	S.E.±		0.51
	C.D. (P=0.01)		2.18

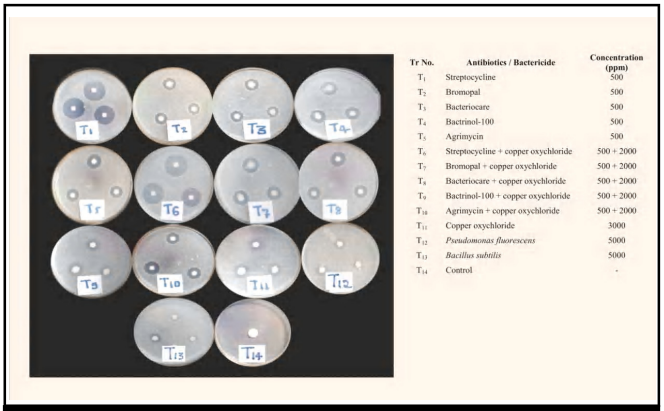

Fig. 4 : *In vitro* efficacy of different chemicals and boi agents against growth of *Xanthomonas campestris* pv. *viticola*

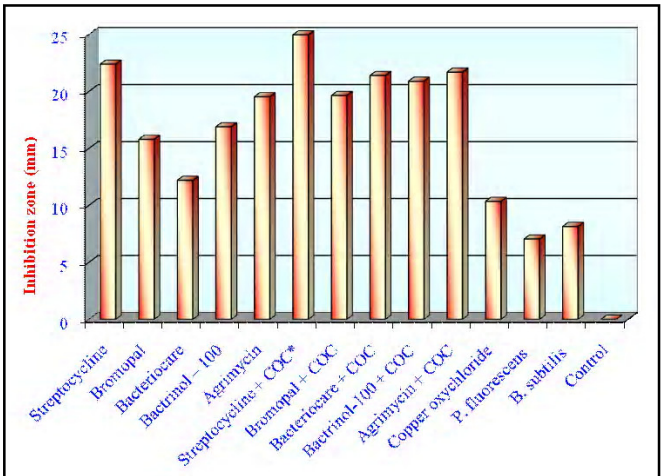

Fig. 5 : *In vitro* efficacy of different chemicals and bioagents against growth of *Xanthomonas campestris* pv. *viticola*

Bacteriocare 500 ppm plus copper oxychloride 2000 ppm (21.40 mm). Similar results were enumerated by Chauhan and Vaishnav (1980), Sharma *et al.*(1981), Rameshchand *et al.* (1991) and Namasivayam (1969). Among the bio-agents, the *Bacillus subtilis* was found to be more effective in inhabiting the growth (8.20 mm) of the pathogen than *Pseudomonas fluorescens* (7.05 mm). This was reported by earlier workers like Chen *et al.* (1990), Sakthival *et al.* (1986), Sivamani *et al.* (1989), Gallardo *et al.* (1989) and Karuna (1993).

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