STUDIES ON BACTERIAL LEAF SPOT OF GRAPE CAUSED BY *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye IN NORTHERN KARNATAKA

Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

MASTER OF SCIENCE (AGRICULTURE)

In

PLANT PATHOLOGY

By

SHIVANANDA JAMBENAL

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD – 580 005

SEPTEMBER, 2008

Advisory committee

DHARWAD

SEPTEMBER, 2008 **(M. R. RAVIKUMAR)**
CHAIRMAN

Approved by :
Chairman : ______________________________
(M. R. RAVIKUMAR)

Members : **1.** __________________________
(YASHODA R. HEGDE)

2. __________________________
(SHAMARAO JAHAGIRDAR)

3. __________________________
(K. RAMACHANDRA NAIK)

CONTENTS

Sl. No.	Chapter Particulars
	CERTIFICATE
	ACKNOWLEDGEMENT
	LIST OF TABLES
	LIST OF FIGURES
	LIST OF PLATES
1.	INTRODUCTION
2.	REVIEW OF LITERATURE
	2.1 Symptomatology
	2.2 Survey of bacterial leaf spot of grape caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye in Northern Karnataka
	2.3 Isolation, identification and pathogenicity studies
	2.4 *In-vitro* evaluation of different chemicals alone and their combination against *X. campestris* pv. *viticola*
	2.5 *In-vivo* evaluation of different chemicals alone and their combination against *X. campestris* pv. *viticola*
3.	MATERIAL AND METHODS
	3.1 General procedures
	3.2 To survey for bacterial leaf spot of grape in major grape growing area of Northern Karnataka
	3.3 Symptomatology
	3.4 Isolation of *Xanthomonmas campestris* pv. *viticola* from infected grapes leaves
	3.5 *In-vitro* evaluation of different chemicals alone and their combination against *X. campestris* pv. *viticola*
	3.6 *In-vivo* evaluation of different chemicals alone and their combination against *Xanthomonas campestris* pv. *viticola*
4.	EXPERIMENTAL RESULTS
	4.1 Survey for bacterial leaf spot of grape caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye in Northern Karnataka
	4.2 Symptomatology
	4.3 Isolation of *Xanthomonas campestris* pv. *viticola* from infected grape leaves
	4.4 *In-vitro* evaluation of different chemicals alone and their combination against the growth of *Xanthomonas campestris* pv. *viticola*
	4.5 *In-vivo* evaluation of different chemicals alone and their combination against the growth of *Xanthomonas campestris* pv. *viticola*

Contd…..

Sl. No.	Chapter Particulars
5.	DISCUSSION
	5.1 Survey for bacterial leaf spot of grapes caused *Xanthomomas campestris* pv. *viticola* (Nayudu) Dye in Northern Karnataka
	5.2 Symptomatology
	5.3 Isolation, identification and pathogenicity studies
	5.4 *In-vitro* evaluation of different chemicals alone and their combination against growth of *Xanthomomas campestris* pv. *viticola*
	5.5 *In-vivo* evaluation of different chemicals alone and their combination against growth of *Xanthomomas campestris* pv. *viticola*
6.	SUMMARY AND CONCLUSIONS
	REFERENCES

LIST OF TABLES

Table No.	Title
1.	List of different chemicals alone and their combinations used at different concentrations to study their effect on the growth of *Xanthomonas campestris* pv. *viticola* under *in-vitro* and *In-vivo*
2.	Survey for bacterial canker of grapes in Northern Karnataka
3.	Morphological and biochemical characteristics of *Xanthomonas campestris* pv. *viticola* causing bacterial leaf spot of grape
4.	Effect of chemicals and their combination on the growth of *Xanthomonas campestris* pv. *viticola* under *In-vitro* condition
5.	*In-vivo* evaluation of different chemicals alone and their combination against of *Xanthomonas campestris* pv. *viticola*
6.	Number of leaves infected per leaflet before and after spray
7.	Severity of disease before and after spray
8.	Yield and yield parameters
9.	Economic evaluation of bactericides, antibiotics and bioagents alone and their combination against *Xanthomonas compestris* pv. v*iticola* under field condition

LIST OF FIGURES

Figure No.	Title
1.	Effect of different chemicals, bioagents alone and their combination on the growth of *Xanthomonas campestris* pv. *viticola* under *In-vitro* condition
2.	*In-vivo* evaluation of different chemicals alone and their combination against of *Xanthomonas campestris* pv. *viticola*
3.	Number of leaves infected per leaflet before and after spray
4.	Severity of disease before and after spray
5.	Yield and yield parameters
6.	Yield and yield parameters

LIST OF PLATES

Plate No.	Title
1.	Survey on severity of bacterial leaf blight of grape in Northern Karnataka during 2007-08
2.	Symptomatology of bacterial leaf spot of grapevine
3.	Isolation of *Xanthomonas campestris* pv. *viticola* on nutrient agar
4.	Identification of causal organism
5.	Pathogenicity studies
6.	Effect of chemicals and their combination on the growth of *Xanthomonas campestris* pv. *viticola* under *in-vitro* condition
7.	*In-vivo* evaluation of different chemicals alone and their combination against the growth of *Xanthomonas campestris* pv. *viticola*

1. INTRODUCTION

Grape (*Vitis vinifera* L.) is among the oldest plants (90-95 million years) on earth, existing almost at the time when dinosaurs flourished as evidenced by the recent discoveries from western Kazakhaton (Shanmugavelu, 2003). It is an important temperate fruit of the world native to Europe. It is also cultivated in tropical and sub-tropical regions of the world. It is one of the important horticultural crops grown in India. In India, grapes were introduced into the northern parts from Iran and Afghanistan by Muslim invaders in 12th century. Later it spread to south India in 1338.

The white grapes (*Vitis vinifera* L.) originated between Caspian sea and Black sea. The grape found its first home in Armenia. Details of its cultivation figured in 4th dynasty of Egypt (2440 BC) indicating that the grape was known to man from early days and now the cultivation has spread over major continents. It is extensively grown in Europe (Spain, France, Italy, Portugal, Yugoslavia), North America (USA, Mexico), South America (Argentina, Chile), Africa (Morocco, Algeria, Nigeria), USSR, Australia and Asia.

Grape is utilized in several ways both in fresh and processed forms *viz.,* wine, raisin, juice, jam and jelly. It is rich in energy giving sugars particularly glucose, fructose and contains many useful minerals. Hence, it is also a rich source of vitamin A and good source of biflorohoids known to be useful in condition as pulpusa, capillary edema, radiation damage *etc.* India exports a large quantity of fresh grapes valued Rs. 110 crores annually to different countries around the world (Anon., 2004b).

In recent years, grape vine cultivation is becoming more and more popular, because of its phenomenal yield potential and early bearing habit. It is found to be one of the most remunerative farming enterprises in recent years. North-west frontier and Baluchistan were the principal grape growing regions in undivided India. The partition of country in 1947 necessitated growing grapes indigenously since imports of grapes into India from these areas and Afghanistan was restricted. Though, selling grapes was regarded as hardship by vendors at that time but later it proved to be a boon for the country and cultivation took first root in Southern and then in Western parts of the country.

In the tropical belt of this country, the vines remain evergreen throughout the year and do not enter dormancy as in the temperate zones. The Deccan plateau, has unique climatic factors yielding two crops of grape per annum, by pruning twice a year as against the practice of single pruning done in all temperate grape growing countries of the world. Thus, tropical viticulture has had its birth in India and the country has ventured to produce quality wine for international market at competitive price from Bhokri and Muscat Hamberg, Bhokri and Cheema Sahebi in Tamil Nadu and Maharashtra, respectively.

Grape growing in North India received impetus from its success in South India. Commercial cultivation in North India dates back to early sixties when Pusa seedless variety was released by IARI, New Delhi. During this period, the State Government of Punjab has a thrust to its cultivation by distributing Anab-e-shahi cuttings in 1962, which unfortunately did not pick up. However, later about one lakh cuttings of perlette was imported from California which proved quite successful in commercial cultivation.

Currently, the world production of grapes is over 6.5 million tonnes annually occupying more than 10 million ha in different countries. Area under grapes in India is about 57.80 ha with production of 1.47 million tonnes. Among the various states of India, Southern and Western states cover 88.00 per cent of total area. Maharashtra has the largest area followed by Karnataka, Punjab, Andhra Pradesh, Tamil Nadu and Haryana. Maharashtra stands first occupying the largest area of 41,400 ha under grape cultivation accounting for about 71.5 per cent of total area and 80.00 per cent of the production of grapes in the country. Karnataka state has the distinction of cultivation grapes under tropical conditions and is the second largest area under grapes next only to Maharashtra. At present, the total area under grapes in Karnataka is 9721 ha spread across Bagalkot, Bangalore, Belgaum, Bijapur, Chikmagalur, Raichur, Kolar, Koppal district (Anon., 2007). The major cultivated varieties are Bangalore Blue, Anab-e-shahi, Dilkush, Thompson Seedless and its mutants, Sharad Seedless. The total production of fresh/table grapes in Karnataka is 1,67,044 tonnes with a

productivity of 17.20 tonnes per ha (Vasantha Kumar, 2007). The varieties such as Thompson Seedless and its mutants like Sonaka, Manik Chaman, Tas-E-Ganesh and Sharad Seedless are predominantly grown in the northern parts of Karnataka comprising Bijapur, Bagalkot, Belgaum, Raichur, Koppal contributing to 60.00 per cent of the states grape production.

In India, bulk of production (78%) is used as fresh/table grapes followed by raisin (17.20%). Especially, the northern parts of Karnataka are known for producing high quality raisins. The raisin production is concentrated in Bijapur district, with Thompson Seedless variety which is best suited for raisin making TSS (total soluble solids). It is estimated that annually about 30,000 tonnes of raisin is produced in Bijapur district alone (Adsule *et al*., 2007).

The important grapes growing districts of Karnataka are Bangalore, Kolar, Bagalkot, Bijapur, Gulbarga, Raichur, Koppal and Belgaum. In the beginning viticulture in south India was promoted by French priests for producing local wines in celebration of mass but its commercialization is relatively recent with discovery of Anab-e-shahi variety by Mr. Shanker Pillay in 1930 from bungalow of Nawab Baquer Alikhan. The variety performed very well in Hyderabad providing initial boost to Indian viticulture and revolutionizing grape industry in south India. This success aroused interest among the grape growers in other states of country as well and commercial cultivation picked up slowly in Karnataka with variety Bangalore blue. Though, the crop is of high commercial value.

The low productivity is mainly attributed to abiotic stresses besides, attack by insect pests and pathogens leading to heavy crop losses. Grape suffers from many diseases like powdery mildew, downy mildew, anthracnose, bacterial leaf spot *etc.* Among them, bacterial leaf spot caused by *Xanthomonas campestris* pv. *viticola* is an important disease. It has become more serious in major grape growing areas of Northern Karnataka.

Bacterial leaf spot of grape was noticed for the first time on *Vitis vinifera* cv. Anab-e-shahi at Tirupati (Andhra Pradesh) during 1960 (Nayudu, 1972). He studied the etiology of the disease and identified the casual organism as *Pseudomonas viticola.* But, later it was renamed as *Xanthomonas campestris* pv. *viticola* (Dye, 1978). The disease appeared in epihytotic form during 1984 in September prune vineyards in Sangli and Solapur districts of Maharashtra on cv. Thompson seedless (Patil, 1988). Yield loss was estimated approximately 60 to 70 per cent (Chand and Kishun, 1990a).

The disease is exemplified by the production of water soaked lesions on leaves especially on the veins and vein lets leading to leaf necrosis. In severe cases, the pathogen also infects inflorescence, berries and tender shoots, cankerous lesions on the canes and causing considerable loss in yield and quality of the produce.

Regular inspection of vineyard, destruction of infected plant material, use of disease free cuttings, curbing on excess use of water and late October pruning are recommended for the management of this disease and chemical management was also suggested with spray of antibiotics @ 300 ppm starting from two leaf stage upto 70 days at an interval of 15 days (Chand and Ramakishuna, 1988).

Ravikumar *et al.* (2002) reported spray of streptomycin sulphate or streptocycline @ 500 ppm, sprays at 20 days interval for effective management of the bacterial leaf spot in grape gardens of Northern Karnataka. Presently, the bacterial canker of grape ha become a regular problem in the early pruned (September) vineyards in the areas of Maharashtra, North Karnataka and Andhra Pradesh. Hence, keeping these points in view, the present investigations were envisaged with the following objectives.

1. To survey for bacterial leaf spot in major grape growing areas of Northern Karnataka.
2. To isolate, identify the causal agent and pathogenicity studies.
3. To screen available antibiotics and chemicals against *Xanthomonas campestris* pv. *viticola* under laboratory conditions.
4. Integrated management of bacterial leaf spot of grape in field condition.

2. REVIEW OF LITERATURE

Viticulture in general encounters a major threat from some important diseases throughout the world irrespective of variable cultivation pattern and climate. Now-a-days, bacterial leaf spot of grapes (*Vitis vinifera* L.) caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dey is also one of the important diseases not only to downy mildew, powdery mildew and anthracnose causing serious damage to growth and production of grape vineyard, especially northern part of Karnataka. The disease is distributed throughout temperate, tropics, subtropical regions including India. The literature underlying the objectives in the present investigation are briefed as under.

2.1 Symptomatology

Chand and Kishen (1990a) described symptoms of bacterial leaf spot of grapes caused by *Xanthomonas campestris* pv. *viticola* as angular, raised and cankerous on the leaf blade, petiole and canes. Severe infection lead to the death of leaves and affected the growth of canes.

Chand and Kishen (1990b) reported that the symptoms, in advanced stages of infection lead to stunting, cracking and irregular growth of canes. On the berries lesions are brown to black, cankerous and severely infected berries are small and shriveled.

Malavolta *et al.* (1994) described the symptoms of bacterial canker of grape caused by *Xanthomonas campestris* pv. *viticola* as small, dark and angular leaf spots in a vineyard located in Petrolina, Pernmbuco, Brazil. Later, spots may coalease and dry up, causing necrotic areas and leaf blight. The midribs may also show a discolouration. Cankers were observed in petiole, stems and rachis and also observed on bunches.

Araujo and Robbs (2000) described the symptoms as necrotic and angular spots on leaf blades, petiole and vein discolourations, extensive deep cankerous on the branches.

2.2 Survey of bacterial leaf spot of grape caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye in Northern Karnataka

Patil (1988) reported the disease in epiphytotic form during 1984 in Sangali, Solapur districts of Maharashtra on Thompson Seedless grape. All nurseries surveyed produced infected planting material, although the percentage produced varied in different areas. In 1990, nine of the 10 locations studied had infection levels of 85 to 100 per cent. The disease incidence of cuttings collected in October was much less than that of April cuttings as reported by Ramesh Chand *et al.* (1991).

Chand and Patil (1993) carried out the survey in Maharashtra and North Karnataka and reported that the disease incidence on the grape cultivar Thompson seedless was highest when pruning took place during 1st to 15th September.

2.3 Isolation, identification and pathogenicity studies

2.3.1 Isolation

Hayward (1983) studied the bacterium (*Xanthomonas campestris* pv. *viticola*) isolated from the samples (leaves, petioles, canes and bunches) on nutrient agar by streak plate method. Maximum populations (46 colonies/plate) recovered from canes and petioles after three days of incubation. Very low population (2 colonies/plate) was recovered from infected leaf.

Mariano and Gama (2005) observed that the bacterial colonies were yellow, mucoid, slimy, glistening, convex and round in shape, isolated from infected leaves in case of *Xanthomonas campestris* pv. *viticola* in grape.

Viana (2006) found that, bacterial colonies were deep yellow, slimy, irregular to round in shape, when isolated on yeast dextrose calcium carbonate agar medium (YDCA) from infected leaves and cane of bacterial blight of grapes.

2.3.2 Identification of causal organism

2.3.2.1 Morphological, physiological and biochemical characters

Schaad and White (1974) found that the bacterial colonies on nutrient agar are white, round, smooth, glistening with entire margin. On SX agar produced a clear starch digestion zone (3 – 4 mm). Bacterium is gram negative, rod shaped with rounded ends, motile by single polar flagellum and $0.4 - 1.2 \times 2 – 3$ µm in size.

Malavolta *et al.* (1999) described symptoms of bacterial leaf spot of grape as dark and angular leaf spots that coalease causing necrotic areas and leaf blight. Cankers were often observed on petioles. Further, based on characterized by biochemical, cultural, physiological and pathogenicity tests identified as *Xanthomonas campestris* pv. *viticola*.

Araujo and Robbs (2000) reported that the new bacterial disease on grapevine (*Vitis vinifera*) in Brazil and causing severe damage to local crops. Further, they identified the causal agent by morphological, physiological and biochemical test as *Xanthomonas campestris* pv. *viticola*.

Halfeld-Vieira and Nechet (2006) studied the symptomatology of bacterial canker of grape in Rosaima, Brazil, exhibited stem canker and leaf necrosis symptoms based on morphological and biochemical analysis identified the isolate as *Xanthomonas campestris* pv. *viticola*.

2.3.2.2 Pathogenicity

Lenka *et al.* (1997) studied the pathogenicity of the black rot pathogen by employing two different methods. Plants inoculated with isolates Xc-1, Xc-2, Xc-3 and Xc-4 showed typical symptoms of black rot within 10 days after inoculation, with blackening of leaves and chlorosis of the margins (V-shaped lesion).

2.3.2.3 Hypersensitive reaction

Graham (1982) noted that isolates *Xanthomonas phaseoli* var. *sojense* from soybean elicited hypersensitive reaction on tobacco leaves. Khan (1989) for the first time used hypersensitive reaction on tobacco for the detection of *Xanthomonas campestris* pv. *vignicola*.

2.3.2.4 Inoculation methods

Most important aspect of inoculation is to test for pathogenicity and the experimental induction of disease i.e., proving Koch's postulates. Unlike the fungi, most of the phytopathogenic bacteria are incapable of direct penetration of cutinized layers of cells of the host. The main point of entry are non-cutinized areas such as root hair and some natural openings like stomata, hydathodes *etc.* The majority of infections by bacteria occur through wounds or punctures.

Different methods have been used to inoculate plants with bacteria in the field or in the glasshouse. The choice of the method depends on the type of host plants, plant part and age of bacterium. The concentration of the phytopathogenic bacterial cells and the follow up environment are also important. Spraying is one of the methods most commonly used. It is particularly used in diseases where the bacteria enter through natural openings such as stomata, water pores and nectaries.

Younger leaves of sesame which are more susceptible to *X. sesame* (Sabet and Dowson) Dye had more stomata, higher nitrogen and moisture content than older ones (Shukla *et al.*, 1976).

Koizumi (1976b) studied that the bacteria multiplied soon after inoculation in wounded tissues and intercellular spaces when detached leaves were incubated at 6^0 to 25^0C but not at 40^0C. Further, the period from inoculation to multiplication was influenced slowly by temperature and not by inoculum density.

Hazel and Civerolo (1980) reported that the spray infiltration was the most rapid method of inoculation for strawberry angular leaf spot disease caused by *X. fragariae.*

Bora and Addy (1982) found that the plants showed disease symptom earlier when inoculated at higher dose (200 – 2000 cells). Deep inoculation (5 mm) was better than shallower (2 mm).

Gupta and Chakravarti (1983) studied the different inoculation techniques in cabbage for development of infection due to *X. campestris* pv. *campestris.* The minimum inoculum concentration needed for development of disease was 10^6 cells per ml. The bacterial population reached maximum on third day of inoculation in case of injection infiltration method, whereas it reached maximum on eighth day of inoculation on pricking with multineedle method.

Hayward (1983) reported inoculation of both bacterium (*X. campestris* pv. *viticola*) on leaves (cv. Thompson Seedless), by pinprick method was found to produce typical disease symptoms within 10 days and 20 days in petioles and canes. Typical water soaked symptoms appeared around the margin of pinprick, which gradually increase in size and transformed into angular lesions.

Vajawat *et al.* (1985) showed that minimum concentration (1×10^5 and 1×10^4 cells per ml) of *P. syringae* pv. *sesame* (Malkoff) Young Dye and Wilkie was necessary for symptom development. Inoculations were carried out by spray and carborandum method, on sesamum.

Xanthomonas campestris inoculated on different cauliflower varieties with vein and hydathode inoculation, the optimum concentration (4.3×10^8 cells/ml) produced symptoms. Pinpricking of vein was found better than spraying higher infection (Bandyopadhyay and Chattopadhyay, 1985).

Lapwood and Read (1986) reported different methods, wooden tooth pick rubbed in bacterial slime was more successful in establishing infection than drip method. Injection with hypodermic needles was reliable technique for infection. Detached stems technique was employed with consistent results, while studying the black leg of potato caused by *E. carotovora* sub. sp*. atroseptica* (Van Hall) Dye.

Manicom (1986) stated that bacterial black spot of mango caused by *X. campestris* pv. *mangiferae indicae* was found to be essentially a wound pathogen. Punctured leaves with multiple needle device, sprayed to run-off with bacterial suspension using an automizer exhibited good symptoms within two weeks.

Under greenhouse condition, hypodermic inoculation was found better than hand rubbing, when inoculated to wheat by *X. campestris* pv. *translucens* (Akhtar and Aslam, 1988).

Chand and Kishun (1991) studied that the susceptibility of nodal leaves found in ascending order from first to fourth node. Among these fourth nodal leaf is highly susceptible. The susceptibility is found in decreasing order from 5th leaf onwards and disease intensity is minimum on 9th leaf.

Rameshchand and Ramkishun (1991) reported that the leaves were particularly susceptible to the disease at 60 to 75 days old and susceptibility was also varied with leaf position.

Rameshchand and Ramkishun (1991) described the different methods of inoculation for grapevine with *X. campestris* pv. *viticola.* They found that spraying inoculum on mechanically damaged leaves was found to be most effective, resulting in 100 per cent disease incidence and 53 per cent severity. Spray inoculation alone resulted in 72.5 per cent and 44.5 per cent disease incidence and severity respectively. Maximum disease intensity occurred after 15 days incubation period.

2.4 *In-vitro* evaluation of different chemicals alone and their combination against *X. campestris* pv. *viticola*

Thirumalachar *et al.* (1956) reported *in vitro* efficiency that chloramphenicol, streptomycin sulphate, terramycin and aureomycin inhibited *Xanthomonas phaseoli* pv. *sojense*.

Chakravarti and Rangarajan (1966) studied *in-vitro* effect of streptocycline on seven species of *Xanthomonas*, six species of *Erwinia* and one each of *Pseudomonas, Corynebacterium* and *Agrobacterium.*

Desai *et al.* (1967) reported maximum inhibition against *Xanthomonas* and *Pseudomonas* at 250 ppm of streptocycline.

Namasivayam (1969) found that streptocycline gave the best result followed by streptomycins in inhibiting *X. campestris*.

Chen *et al.* (1972) reported that the effect of streptomycin and several minerals on *E. mangiferae*, *X. oryzae* and two bacterial isolates from sunflower.

Streptomycin, tetracycline and streptocycline were found effective against *X. bataticola* of piper betla (Nema *et al.*, 1975).

Chauhan and Vaishnav (1980) found that the best control of *Xanthomonas campestris* pv. *oryzae* with streptocycline and copper containing compounds under *in-vitro* and *in-vivo* conditions.

In-vitro evaluation of eight chemicals against *X. vesicatoria* by paper disc and turbidometric method was studied by Sharma *et al.* (1981) and described the effectiveness of combination of streptocycline and copper sulphate.

Sharma *et al.* (1982) showed effectiveness of streptocycline alone or with copper sulphate, agramycine, mancozeb were most effective against *X. campestris* pv. *vesicatoria*.

Venugopal (1983) studied *in-vitro* sensitivity of different isolates to antibiotics by paper disc method against *X. campestris* pv. *mangiferae indicae* isolate from local, Lalbaugh, Raspuri and Bappukai by Paushamycline and streptomycin @ 100 and 250 ppm were found effective.

Ashrafuzzaman (1987) found that the effectiveness of 11 chemicals *in-vitro* and *in-vivo* against bacterial blight of paddy caused by *X. campestris* pv. *oryzae*. Phytomycin, streptomycin, bladticidin and kasumin inhibited the growth of bacteria *in-vitro* and produced good inhibition zones.

Mahto *et al.* (1988) achieved maximum inhibition with streptomycin among two antibiotics and eight fungicides tested against *X. campestris* pv. *oryzae*. Streptocycline was inhibitory @ 10, 100 and 200 ppm, while streptomycin, MEMC and foltap were inhibitory only at higher concentrations. Bacterinol-100 was reported to be effective against bacterial leaf blight of rice caused due to *X. oryzae* when tested *in-vitro* (Anon., 1990).

2.4.1 Effect of antagonistic microorganisms

Sakthival *et al.* (1986) reported that the *in-vitro* antagonism between strains of *P. fluorescens* and *X. campestris* pv. *oryzae* and *Pseudomonas syringae* pv. *phaseolicola*.

Gallardo *et al.* (1989) reported the *in-vitro* inhibition of *P. solanacearum* BC-8 strain of *P. fluorescens*. The growth of *P. solanacearum* on agar was inhibited by inoculation with *P. fluorescens* (Str BC-8) or by application of *P. fluorescens* culture extracts. It was found that the extracts contained rod shaped particles with a helical structure 150 mm long and 25 mm in diameter. These were interpreted as bacteriocins and designated as fluocin BC-8. Bacterioxin activity was found to be associated with plasmids since plasmid DNA was not detected electrophoretically.

Sivamani *et al.* (1989) reported that the toxicity of *P. fluorescens* towards banana strain of *P. solanacearum* and *X. campestris* pv. *oryzae*.

Chen *et al.* (1990) reported that *Enterobacter cloacae* B8 from rice and *B. subtilis* B826 from squash were strongly antagonistic to *X. campestris* pv. *oryzae* and also showed activity against many other phytopathogenic bacteria.

Chand *et al.* ((1991b) reported antagonistic activity of *Erwinia herbicola* and *Bacillus subtilis* against *Xanthomonas campestris* pv. *viticola in-vitro*.

Karuna (1993) also noticed effectiveness of *Pseudomonas fluorescens* and *B. subtilis* were effective against bacterial wilt of tomato under *in-vitro* and *in-vivo* conditions.

2.5 *In-vivo* evaluation of different chemicals alone and their combination against *X. campestris* pv. *viticola*

Ercolani (1968) reported the incidence of *X. vesicatoria* and *Corynebacterium michigananse*, was 19 and 16 per cent, respectively by spraying with agrimycin-100 followed by phenyl mercuric acetate (8 and 13%) and acetic acid (8 and 2%), respectively. Mercuric chloride from 0 and 14 per cent and hot water to zero and six per cent. Mathur *et al.* (1973) found that seed dressing (3 g/40 kg seed) and a spray of agrimycin-100 on *X. malvacearum* of cotton.

Spraying 0.2 per cent copper oxychloride was the best treatment for the control of *X. malvacearum* (Singh and Verma, 1973).

Chakravarti *et al.* (1976) stated that the bacterial blight of cowpea could be controlled by spraying agrimycin-100 @ 250 ppm, thrice at an interval of 10 days and recorded upto 35 per cent increase in yield over control.

Evaluation of antibiotics and fungicides against bacterial blight of guar (*Cyamopsis tetragonoloba*) was carried out by Gupta (1977). The best control of the disease was obtained with streptocycline @ 100 to 250 ppm and agrimycin-100 @ 100 to 500 ppm.

Jeyachandran and Shanmugam (1979) reported that combined application of agrimycin-100 (0.01%) + copper oxychloride (0.1%) effectively reduced secondary infection of *X. malvacearum* and enhanced yield. Rajpurohit and Lodha (1982) also reported that three sprays of agrimycin-100 + blitox-50 reduced the disease intensity to 46.49 per cent and increased the yield of seed cotton by 26.54 per cent. Alcaraz (1982) revealed that tribasic copper sulphate (0.5%) gave the best result and intensity was reduced by the addition of agrimycin-100 @ 0.12 per cent or Manzate-D (Maneb + Zineb) @ 0.2 per cent when sprayed on grape fruit (*Citrus paradisi*) and against citrus canker (*X. campestris* pv. *citri*).

Kishun *et al.* (1979) obtained better control of bacterial leaf spot of Zinnia caused by *Xanthomonas negromaculans* f. sp. *zinniae* by three applications at 10 to 15 days intervals of streptomycin (500 ppm) and agrimycine-100 (500 ppm).

Chauhan and Vaishnav (1980) observed that the spraying of streptocycline and copper containing compounds gave better control against *X. campestris* pv. *oryzae* under field conditions.

Ganacharya (1980) reported that the Blitox-50 and streptomycin + copper sulphate was effective in reducing the disease intensity of bacterial pustule of soybean.

Preblosoom sprays of streptocycline at 200 and 300 ppm at 20 days interval reduced the fruit infection from 40 to 15 per cent for the control of fruit canker of mango incited by *P. mangiferaeindicae* (Bose and Chokha Singh, 1980).

Raju *et al.* (1980) reported that best field control of *X. campestris* pv. *visicatoria* on capsicum by agrimycin-100 + copper oxychloride and copper oxychloride + streptomycin.

Balaraman and Purushothman (1981) found that spraying of agrimycin-100 (streptomycin), Bordeaux mixture and copper oxychloride gave the better control against *X. campestris* pv. *citri*.

Three protective sprays with chemicals including streptocycline + copper sulphate, streptocycline, agrimycin-100, blitox 50 WP and other chemicals on artificially inoculated plants reduced the disease intensity significantly, but agrimycin-100, streptocycline alone, combination of streptocycline + copper sulphate and Dithane M-45 proved most promising against *X. vesicatoria* causing bacterial leaf spot of chilli (Sharma *et al*., 1981).

Kishun and Sohi (1982) found that three application of agrimycin-100 was effective in reducing infection by *X. campestris* pv. *vesicatoria*

Sharma *et al.* (1982) evaluated the effectiveness of streptomycin alone or with copper sulphate, agrimycin-100, Dithane M-45 against *X. campestris* pv. *vesicatoria* in field test.

Shekhawat *et al.* (1982) reported that agrimycin-100 + captan and streptomycin + captan were effective against elimination of *X. campestris* pv. *campestris* from seed causing black rot of cabbage.

Wet (1982) reported that seven applications of copper oxychloride between 1st October and 27th January at 600 g per 100 litre water gave 76 and 66 per cent healthy fruits with only 5 and 26 per cent fruits from unsprayed control, respectively against *X. campestris* pv. *mangiferaeindicae*..

Mary and Mathew (1983) found that pencillin @ 500 ppm was inhibitory to the growth of bacterial blight of rice pathogen in *in-vitro* conditions.

Padagnur and Basavaraj (1983) reported that effectiveness of copper oxychloride and streptomycin sulphate and to a lesser extent, copper oxychloride alone significantly reduced bacterial blight of cotton caused by *X. campestris* pv. *malvacearum*.

Plessis (1983) reported that the regular sprays with 200 g per 100 lit gave better control against *X. campestris* pv. *pruni* causing bacterial leaf spot on plums.

Ramkishun and Sohi (1984) also tried combined application of copper oxychloride and agrimycin-100 and found that copper oxychloride alone reduced bacterial canker on mango.

Gaur *et al.* (1984) reported that agrimycin-100 at 200 ppm + Bavistin @ 1000 ppm used as seed dressing and agrimycin-100 alone gave good control against *X. campestris* pv. *phaseoli*.

Ramkishun and Sohi (1984) reported that four sprays starting from May to August of bavistin (1000 ppm) or bavistin + agrimycin-100 (1000 + 100 ppm) at monthly interval were found significantly effective in reducing the intensity of bacterial canker from 53.33 to 9.33 per cent.

Srivastava and Bais (1985) found that the seed treatment with thiram combined with foliar sprays of streptomycin gave effective control against *X. campestris* pv. *glycines*.

Gitaitis *et al.* (1986) stated that spraying of copper and copper + maneb mixtures controlled leaf blight and stem canker symptoms of cowpea and further significantly increased the yields.

Krishna and Nema (1986) found that the best control of *X. campestris* pv. *citri* was achieved with streptomycin @ 500 ppm with four spray schedule followed by paushamycin, plantomycin, Bordeaux mixture and agrimycin.

Thapliyal and Dubey (1986) reported that the combination of a antibiotic with a copper fungicide generally gave improved control of *Xanthomonas campestris* pv. *sojense* on soybean and the highest yields were obtained with agrimycin-100 + Blitox-50 (copper oxychloride).

Spraying with bactrinol (1000 ppm) gave better control of citrus canker and was found to be advantageous over bordeaux mixture and developed spray schedule as two before the onset of the monsoon (April to May), two during monsoon (July to September) and two after rainy season (November to December) with 15 days interval (Balaraman, 1987).

Thombre *et al.* (1989) obtained better control of bacterial pustule of soybean by spraying with mixture of streptocycline + copper oxychloride.

Rao (1990) reported that the protection of tomato (*Lycopersicon esculentum* L.) bacterial disease (*P. solanacearum*) was possible by seed dressing and spraying with 0.1 per cent of bactrinol-100.

Kore and Dhutraj (1991) reported that seed treatment by soaking soybean for 20 minutes in chemical solutions of carbendazim, thiram, and streptocycline eliminated *X. campestris* pv. *glycines* from the seeds.

Ramesh Chand *et al.* (1991) reported about intensity of bacterial canker on the grape cultivars Thompson Seedless, Tas-e-Ganesh and Sonaka was highest when pruning took place during 1 to 15 September (76.8%) and yield were lowest. Disease was <5.6 per cent in vineyards pruned after 10 October. Pruning the vineyard from the second week of October and onwards gave the best result in the grape production areas of Maharashtra and North Karnataka.

Mandge *et al.* (1992) reported that the *X. campestris* pv. *vesicatoria* was effectively controlled with three sprays of agrimycin + copper oxychloride + carboryl.

Ramesh Chand *et al.* (1992) observed in field trials, copper oxychloride, followed by Bordeaux mixture were found effective to reduce intensity of bacterial canker on grape. But were much less effective in areas with high and frequent rainfall, prophylactic sprays of these chemicals every seven days from pruning until the onset of dry weather are suggested for low rainfall areas.

Thirumurthi and Agarwal (1992) opined that spraying a combination of streptocycline and copper oxychloride was effective against bacterial pustule of soybean.

Chand and Patil (1993) experimental results confirmed that the pruning from 1st September to 15th September is highly favourable for development of bacterial canker of grape in the most grape cultivating areas of Maharashtra and North Karnataka. This is because of frequent rains during this period that helped disease development and spread. Growers showed prune their vineyards after second week of October, because monsoon generally reduces from this time onwards which is unfavourable for disease development.

Ravikumar and Khan (1995) reported that the seed treatment with streptomycin sulphate or streptocycline for 120 minutes at 300, 400 and 500 ppm eliminated the *X. campestris* pv. *vesicatoria* from tomato seeds.

Chand and Ramakishun (1988) reported that regular infection of vineyard, destruction of infected plant material, use of disease free cuttings, curbing on excess use of water and late October pruning are recommended for the management of bacterial canker of grapes. Chemical management also suggested with spray of antibiotics @ 300 ppm starting from two leaf stage upto 70 days at interval of 15 days.

Ravikumar and Khan (2001) reported that three sprays with streptomycin sulphate (500 ppm) + copper oxychloride (2000 ppm) was found very effective in reducing the disease incidence from 66.70 to 15.75 per cent followed by streptocycline (500 ppm) alone (65.52 to 19.52%).

Ravikumar *et al.* (2002) reported spray of streptomycin sulphate or streptocycline @ 500 ppm, three sprays at 20 days interval for effective management of the bacterial leaf spot of grapes in North Karnataka.

Ravikumar *et al.* (2006) reported that five sprays with bacterinashak (500 ppm) + copper oxychloride (2000 ppm) was found effective in reducing the incidence of bacterial blight of pomegranate (20.20%) followed by streptocycline (500 ppm), copper oxychloride (2000 ppm) (20.70%) when compared to control (73.40%). The maximum yield of 7.66 tonnes per ha in streptocycline (500 ppm) plus copper oxychloride. The untreated check recorded yield of 1.53 tonnes per ha. The maximum cost:benefit ratio of 1:25:1 was recorded in bacterinashak plus copper oxychloride.

Manjula *et al.* (2007) conducted a field trial for the management of bactrinol blight of pomegranate using antibiotics and botanicals. Paushmycin, streptocycline and K-cyclin were effective in suppressing the disease. However, botanicals were moderately effective under field condition in managing the disease.

2.5.1 Yield

Raju *et al.* (1980) reported that the highest yield was obtained with agrimycin-100 + Blitox and copper oxychloride + streptocycline.

Kishun and Sohi (1982) reported that three applications of agrimycin-100 were effective in increasing yield and reducing the intensity of *X. campestris* pv. *vesicatoria* on tomato.

Balaraman and Purushothman (1981) found that spray of agrimycin-100 (streptomycin) against *X. campestris* pv. *citri* resulted in a 22 per cent yield increases over the control.

Chand and Kishun (1990a) reported that importance of the bacterial canker of grape was first visualized in 1983 when it caused substantial loss in yield. During 1986-87, it appeared in the epiedemic form in the September pruned vineyards and yield loss was estimated approximately 60 to 80 per cent.

Chand *et al.* (1991b) reported maximum yield loss (68 – 80%) in the vineyard pruned in the first half of September. In general, disease incidence was reduced when pruning was done after 15th September. Disease intensity was less than 10.8 per cent in all the vineyards pruned during the first half of October.

3. MATERIAL AND METHODS

In the present investigations laboratory studies on isolation, biochemical test, pathogenicity test as well as *in-vitro* studies of different chemicals were carried out at the Department of Plant Pathology, College of Agriculture, UAS, Dharwad and field studies were undertaken at farmers field in Bijapur district during 2007-08. The details of materials used and methodology followed during the course of investigation described hereunder.

3.1 General procedures

3.1.1 Glassware and cleaning

Corning glassware were used for all the experimental studies and kept in the cleaning solution containing 6 g of potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulphuric acid (H_2SO_4) in 1 litre of water for a day. They were cleaned by washing with the detergent followed by rinsing with distilled water.

3.1.2 Sterilization

All the glassware sterilized in a hot air oven at 160^0C for two hours. Both the solid and liquid media were sterilized at 1.1 kg per cm^2 pressure for 15 minutes in autoclaved for all present studies.

3.2 To survey for bacterial leaf spot of grape in major grape growing area of Northern Karnataka

The fixed plot survey was undertaken in three districts of Northern Karnataka *viz*., Bijapur, Bagalkot and Belgaum district during 2007-08. The observations were made on severity (0 – 5 scale) of the disease at periodical time both during April and October pruning periods.

The infected leaves showing the typical symptoms of bacterial spot were collected during *kharif* season from farmers fields of major grape growing areas of Northern Karnataka. Geographically, Bijapur is located in northern dry zone (Zone-3) of Karnataka at N latitude $16^0$59' and E longitude $75^0$43' and altitude (MSL) of 594 m, while Dharwad lies in the transitional belt at N latitude $15^0$15' and E longitude $75^0$07' and altitude (MSL) of 774 m.

3.3 Symptomatology

The infected leaves were collected from major grape growing areas of Northern Karnataka during 2007-08. *Xanthomonas campestris* pv. *viticola* is an incitant of bacterial leaf spot of grape. Symptoms started as production of minute water soaked lesions on leaves especially on the veins and veinlets, irregular to angular and cankerous. In severe case, the pathogen also infects inflorescens, tender shoots as well as cankers lesions on the canes.

3.4 Isolation of *Xanthomonmas campestris* pv. *viticola* from infected grapes leaves

Leaves of grapes showing typical symptoms of bacterial spot caused by *Xanthomonas campestris* pv. *viticola* were collected during the *kharif* season of 2007-08 from major grape growing areas of Northern Karnataka. The bacterium was isolated by extracting the ooze in sterile distilled water taken in test tubes followed by dilution plate technique on nutrient agar.

Small pieces of infected leaves were cut aseptically from the edge of typical spots along with a little portion of healthy tissue. The infected leaf bits were surface sterilized in 70 per cent alcohol or 1 per cent sodium hypochlorite and washed in three series of sterile water to remove traces of alcohol. The infected leaf bits were then suspended in a small test tube containing 3 ml sterilized distilled water for 10 min. When water became slightly turbid due to oozing of bacterial cells from the cut ends of the diseased tissue, the bacterial suspension was serially diluted in 9 ml sterile distilled water. Then one ml of the diluted bacterial cell suspension was poured into sterilized petriplates containing nutrient agar. The plates were rotated gently in clockwise and anti-clockwise direction, so as to distribute the bacterial cell suspension uniformly in the plates and to obtain well separated bacterial colonies. The inoculated plates were incubated at 28^0C for 72 hours. Observations were made for development of well separated light yellow, convex, small bacterial colonies on the nutrient agar medium.

3.4.1 Purification of bacterial culture

The suspected bacterial colonies were picked up with the help of sterilized inoculated loop and streaked onto the surface of yeast extract dextrose calcium carbonate agar (YDCA, Schaad and Stall, 1988). The inoculated plates were incubated at 28^0C for 48 to 72 hours and the observations were made for the development of well separated light yellow coloured bacterial colonies. The purified bacterial colonies were streaked on nutrient agar slants and stored at 5^0C in refrigerator and also in sterile distilled water taken in small culture tubes, by suspending 2-3 loop full of the bacterial culture for future use.

3.4.2 Identification of causal organism

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).

3.4.3 Physiologic and biochemical characters

The physiologic and biochemical characters of the isolate of the bacterium was studied for hydrolysis of starch, gelatin liquifaction, indole production, hydrogen sulphide production, urease production and acid from different sugars *viz*., glucose, mannose, arabinose, galactose, fructose, lactose, maltose, sucrose and alcohol. The tests were conducted as per the methods described by Bradbury (1970) and Schaad and Stall (1988).

a. Utilization of glucose, sucrose, fructose for acid production

The acid and gas production by the isolate was tested by using basal medium supplemented with bromothymol blue. Ten ml of basal medium was dispensed in each test tube. Small Durham's tubes were introduced into these tubes in inverted position. The complete set was sterilized in an autoclave for 15 minutes. To these tubes, filter sterilized carbohydrates i.e., glucose, fructose, sucrose were added @ 0.1 per cent of 24 hours old bacterial culture and incubated at room temperature for 72 hours. Observations were made for gas production in Durhams tube and change in colour of the medium in case of acid production.

b. Utilization of asparagines as sole source of carbon and nitrogen

Asparagine can be utilized both as carbon and nitrogen source by some of the phytopathogenic bacteria and not by others. The test is carried out in the following medium.

Solution 1 : K_2HPO_4, 8 g; KH_2PO_4, 2 g; distilled water, 100 ml.

Solution 2 : $MgSO_4.7H_2O$, 2 g, $FeSO_4$, 0.5 g; NaCl, 1 g; $MnSO_4$, 0.02 g, H_2SO_4, 1 drop; distilled water 100 ml.

Solution 3 : Na_2MoO_4, 0.02 g; distilled water, 100 ml.

Solution 4 : $CuSO_4$ saturated solution in distilled water

Mixed 10 ml of each solution in the order 3, 4, 2, 1 as described above and filtered and added 960 ml distilled water and 2 g per L asparagines. Dispensed the medium in 5 ml quantities in tubes and autoclave. Inoculated with the test bacterium and incubated at 25 – 30^0C and examined for growth. Positive growth indicates utilization.

c. Catalase test

A loopful of 24-48 hours slant growth of the test bacterium was smeared on a slide and covered it with a few drops of 20 volume hydrogen peroxide. The reaction is found to be positive if gas bubbles are produced.

d. Gelatin liquefaction

The following medium (nutrient gelatin) is used.

Peptone, 10.0 g, beef extract 5.0 g, gelatin 20.0 g, water 1000 ml and pH 7.0. Mixed all the ingredients together and heated over a water bath until the gelatin has dissolved. Dispensed the medium in tubes to a depth of about 4 cm and sterilized at 15 lb pressure for 20 min. Cool the tubes and allowed to stand at 20^0C for two days to check the sterility. Inoculated these gelatin columns by stabbing a straight inoculation needle charged with 48 hours growth of the test bacterium. Incubated the tubes at 20^0C. Observe for liquefaction of the gel column at intervals upto one month. The form in which the gel was liquefied by also recorded.

e. Methyl red reaction

The following medium (methyl red broth) is used for the test.

Protease peptone, 5.0 g, glucose C.P., 5.0 g, K_2HPO_4 5.0 g, distilled water 1000 ml and pH 7.0. Dispensed the medium in 5 ml quantities in tubes and sterilized by steaming for three min with for three successive days. Inoculated the tubes with a 48 hours culture of the test bacterium. Incubated the tubes for 7 days at 25^0C and add a few drops of 0.02 per cent methyl red in 50 per cent alcohol to the culture tubes. If a distinct red indicates methyl red positive (methyl red is orange – red at pH 4.5 and lower).

f. Reduction of nitrate to nitrite

Nitrate broth with following composition is used.

Peptone 10 g, beef extract 5.0 g, KNO_3 (nitrite free C.P.) 1 g and distilled water 1000 ml. Dissolved the ingredients by heating in water bath. Dispensed in tubes to a depth of 5 cm and autoclaved. Inoculated the broth with the test bacterium and incubated at 25^0C. Tested for reduction of nitrate upto 15 days at regular intervals. Added a few drops of sulphanilic acid (0.8% in 5 N acetic acid) and dimethyl-alpha-naphthylamine (0.5% in 5 N acetic-acid) to the nitrate broth culture. Nitrite is present if the mixture becomes distinct pink or red. No colour would mean that nitrate is present as such or has been reduced to ammonia and free nitrogen. To confirm either of these two possibilities add few zinc crystals to the above broth-reagent mixture and shake for a few minutes. Nitrates are present without reduction if the broth becomes pink or red. No colour in either of the above two tests would mean that nitrate is reduced to ammonia or free nitrogen.

g. Urease test

The following medium is used.

$NH_4H_2PO_4$ 0.5 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 5.0 g, yeast extract 1.0 g, agar 20.0 g, phenol red 0.012 g and pH 6.8.

Dispensed the basal medium in 90 ml quantities in flasks, autoclaved and cooled to 45^0C. To each flask added 10 ml of 20 per cent filter sterilized urea solution, mixed well and dispensed in tubes in 5 ml quantities. Allowed the medium to solidify in a slanting position. The slants were incubated with the test bacterium, incubated and observations were recorded at regular intervals upto 15 days. If the medium changes from yellow to red, urease production is positive.

h. Indole production

Tryptophan broth medium is used in this test.

Tryptophan or casein digest 10.0 g, NaCl 5.0 g, water 1000 ml and pH 7.0. The medium is dispensed in tubes and autoclaved. To detect indole production the Gnezda oxalic acid test strips are prepared as follows. Soaked Whatman No. 1 filter strips (5×50 mm) in warm saturated solution of oxalic acid solution cooled down. The strips gets covered with oxalic acid crystals and dried at room temperature and used without sterilizing.

Tryptophan broth tubes were inoculated with the test organism and inserted an oxalic acid test strip as directed in H_2S test. Incubated the tubes at 25^0C and observe for colouration of oxalic acid crystals at regular intervals for 14 days. If indole is produced, the oxalic acid crystals on test strip become pink or red.

i. Hydrogen sulphide production

The following medium (peptone water) is used.

Peptone 10 g, NaCl 5.0 g, water 1000 ml and pH 7.0. The medium was dispensed in 5 ml quantities in tubes and autoclaved. To detect H_2S the lead acetate test strips were prepared as follows. Whatman No. 1 filter paper was cut into 5×50 mm strips which are then soaked in warm saturated solution of lead acetate. The strips are then dried, autoclaved and again dried at 60^0C.

The medium in each tube was inoculated with a loopful of 48 hours slant growth of the test bacterium. After inoculation a test strip was inserted in between the plug and inner wall of the tube, so that it hangs just above the broth. The tubes are incubated at 25^0C and observations were recorded at regular intervals upto 14 days. The blackening of test strip indicates liberation of H_2S.

j. Oxidase test

For oxidase test streak a 24 to 48 hours slant growth of the test bacterium on a filter paper saturated with 1 per cent tetramethyl-para-phenylene-diamino-dihydrochloride. The reaction will be positive if a red or purple colour appears within 10 seconds. The reaction will be delayed positive if the colour appears in 10 to 60 seconds.

k) Starch hydrolysis

The medium employed is referred to as starch broth and contains, peptone (10.0 g), beef extract (5.0 g), starch soluble (2.0 g), agar (20.0 g), water (1000 ml) and pH (7.0). Sterilized the medium by autoclaving and pour into sterilized petriplates. Let the medium solidify and spot inoculated the test culture in four plates. The plates were inoculated at 25^0C and test for starch hydrolysis, one plate at a time, after 2, 4, 7 and 14 days as follows. Flooded the agar surface with Lugol's iodine and allow to act a few minutes for development of colourless zone around the bacterial growth.

3.4.4 Hypersensitive reaction on tobacco

The test was carried out to find out whether the bacterial strain is pathogenic or not. Bacterial strains was multiplied in nutrient broth. A loopful bacterial culture was inoculated to 100 ml sterilized nutrient broth contained in Erleyenmayer flask. The inoculated flask was incubated at 28^0C for 72 hours.

The bacterial culture was centrifuged at 33500 g in high speed refrigerated centrifuge for 10 min. The supernatant was discarded and pellets of bacterial strain were collected and suspended in sterile distilled water. The concentration of cell suspension was adjusted to 2×10^8 cfu per ml in spectrophotometer at wave length of 480 nm (Spectronic 20 D. Milton and Roy, USA). The bacterial suspension was injected into intercellular spaces of intact tobacco (*Nicotiana tobacum* var. *samsun*) leaves using hyprodermic needle. The injected plants were kept in glasshouse at 25 ± 2^0C. Leaves which were injected with sterile water served as control. Plants were observed for the development of confluent necrotic lesions in injected leaves during next 24 hours.

3.4.5 Pathogenicity on grape

Pathogenicity test was carried out to find out whether the isolated bacteria was capable of producing typical symptoms of bacterial spot under artificial inoculation condition on grape seedlings or not.

Grape seedlings cv. Thompson Seedless were raised in sterilized soil in nursery in small polythene bags. Thirty days to fourty days old seedlings were transplanted to sterile soil contained in 11 inches plastic pots for the purpose of inoculation of the bacterial isolate.

The strain of *Xanthomonas campestris* pv. *viticola* from grape was multiplied in nutrient broth taken in Earleyenmayer's flask by inoculating a loopful of bacterial culture to nutrient broth. The inoculated flask was incubated at 28^0C for 72 hours. Bacterial suspension was prepared by adjusting cell concentration to 5×10^7 cfu per ml in spectrophotometer at 480 nm (spectronic 20 D, Milton and Roy, USA). The 6 to 8 weeks old grape plants were pre-incubated for 24 hours in humid tent made up of plastic sheets in which humidity was maintained between 60 to 80 per cent before the inoculation.

The leaves of grape plants were slightly injured by insect pin and sprayed with bacterial suspension. The inoculated plants were kept in the plastic tent for two days in which high humidity was maintained by spraying sterile water inside the tent at 25 to 30^0C. The plants were taken out from the plastic tent and kept in glasshouse. Observations were made for the development of symptoms of bacterial spot. Plants similarly, injured and sprayed with sterile water constituted control. The bacterium was reisolated and compared with the original culture of *X. campestris* pv. *viticola* by studying the colony characters and staining and morphology.

3.4.6 Inoculation methods used

a. Vein inoculation

The middle and tertiary veins of the selected healthy tobacco leaves were injected with cells of the stock culture of 48 hours at 28^0C grown on nutrient broth was suspended in sterile distilled water (2×10^8 cfu/ml) with the help of hypodermic syringe. Later, plants were incubated in glasshouse and observed for the development of symptoms.

b. Pin pricking

Leaves of about 4 to 6 weeks old seedlings were selected and washed with tap water. Later leaves were punctured with sterilized pin at several place and the culture prepared as above (2×10^8 cfu/ml) was applied to both the surface of leaves with the sterilized cotton wool and plants were incubated in glasshouse till symptoms developed.

3.5 *In-vitro* evaluation of different chemicals alone and their combination against *X. campestris* pv. *viticola*

The following chemicals *viz.*, streptocycline, bromopal, bacteriocare, Bactrinol-100, Agrimycine, copper oxychloride alone and their combinations were tested at different concentrations (Table 1) against *Xanthomonas campestris* pv. *viticola* by inhibition zone assay method under *in-vitro* conditions.

The bacterium was multiplied by inoculating the cultures in nutrient broth taken in Erleyenmayer's flask. The inoculated flask was incubated at 28^0C for 48 hours. The bacterial culture was seeded to lukewarm nutrient agar. The seeded medium was poured into sterilized petriplates and allowed to solidfy.

The chemicals solution was prepared at different concentrations as given in Table 1. The filter paper discs measuring 5 mm in diameter were soaked in the solutions for 10 minute and transferred onto the seeded medium. The inoculated plates were incubated in the refrigerator at 5^0C for four hours to allow the diffusion of chemical into the medium. The plates were incubated at 28^0C for three days and observed for the production of inhibition zone around the filter paper discs. The bacterium grown on nutrient agar without any chemicals served as control.

3.5.1 Effect of antagonistic organisms on the growth of *Xanthomonas campestris* pv. *viticola*

The efficacy of antagonistic organisms *viz., Pseudomonas fluorescens, Bacillus subtilis* were tested for their inhibitory effect on the growth of *X. campestris* pv. *viticola* by inhibition zone assay method.

A suspension of *X. campestris* pv. *viticola* (2×10^8 cfu/ml) was mixed with molten (50^0C) nutrient agar so as to get a thick growth of the bacterium on the medium. The seeded medium was poured into sterilized petriplates and allowed to solidify. A loopful of the culture of each of the antagonistic bacterium was placed in the centre of the petriplates containing nutrient agar medium seeded with the bacterium. The plates were then incubated at 30^0C for 48 hours.

Observations were recorded for the production of zone of inhibition produced by antagonistic bacteria around *X. campestris* pv. *viticola* colonies by measuring the diameter of the inhibition zone.

3.6 *In-vivo* evaluation of different chemicals alone and their combination against *Xanthomonas campestris* pv. *viticola*

The experiment on management of bacterial canker of grape was conducted in farmer field at Bijapur nearby College of Agriculture, Bijapur during 2007-08. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications on 8 to 10 years old grape vineyards with 1.8 m $\times$ 2.4 m spacing. In each replication and for each treatment, five plants were randomly selected for taking observations. Unsprayed plants served as control and three sprays were taken up at 20 days interval. The required quantities of chemicals were weighed and suitably dissolved in a requisite quantity of water to get desired concentrations. Spraying was done using manually operated high volume (knapsack) sprayer.

The observations were recorded on the severity of the disease on the basis of relative percentage of leaf area covered by the disease using 0 to 5 scale and per cent disease index was worked out using Wheeler (1969) formula. The severity of the bacterial canker on grape was recorded by following 0 – 5 scale of usual reading (Sinha *et al.,* 1988).

Table 1: List of different chemicals alone and their combinations used at different concentrations to study their effect on the growth of *Xanthomonas campestris* pv. *viticola* under *in-vitro* and *in-vivo*

Sl. No.	Antibiotics / Bactericide	Concentration (ppm)
1.	Streptocycline	500
2.	Bromopal	500
3.	Bacteriocare	500
4.	Bactrinol-100	500
5.	Agrimycin	500
6.	Streptocycline + copper oxychloride	500 + 2000
7.	Bromopal + copper oxychloride	500 + 2000
8.	Bacteriocare + copper oxychloride	500 + 2000
9.	Bactrinol-100 + copper oxychloride	500 + 2000
10.	Agrimycin + copper oxychloride	500 + 2000
11.	Copper oxychloride	3000
12.	*Pseudomonas fluorescens*	5000
13.	*Bacillus subtilis*	5000
14.	Control	-

Grade	Per cent of leaf area infected	Reaction
0	No visible infection	Immune (I)
1	1 – 5% infection	Resistant (R)
2	6 – 15% infection	Moderately resistant (MR)
3	16 – 30% infection	Moderately susceptible (MS)
4	31 – 50% infection	Susceptible (S)
5	>50% infection	Highly susceptible (HS)

Per cent disease index (PDI) was calculated as below.

$$PDI = \frac{\text{Total sum of numerical rating}}{\text{No. of leaves examined} \times \text{Maximum grade value}} \times 100$$

3.6.1 Yield and yield parameters

The data on fruit yield and yield parameters obtained from different treatments was also recorded and analysed statistically (Sukhatme and Amble, 1985). The number of bunches produced and number of bunches infected per plant were counted and weight of single bunch was also taken from all the treatments at the end of experiment.

4. EXPERIMENTAL RESULTS

The results of the experiment undertaken during 2007-08 (both *in-vitro* and *in-vivo* studies) on bacterial leaf spot of grapes caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye are presented as under.

4.1 Survey for bacterial leaf spot of grape caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye in Northern Karnataka

The fixed plot survey was taken up during 2007-08 in Northern Karnataka. The observations are made on the severity of the disease during April and October pruning periods.

It is observed from Table 2 that in general, the severity of bacterial leaf spot was more during the April pruned vineyard compared to that of October pruned vineyards.

4.1.1 April pruning

The survey was taken during June to August in the April pruned grapes, the disease was highly severe (grade 5) at Tikota and Chikkapadasalagi having more than 50 per cent infection of grapevine. Whereas, the disease was severe (grade 4) in Jumanal, Bijjarag, having 31 – 50 per cent infection of grapevine and disease was moderately severe (grade 3) at Tidagundi, Lokapur, Athani, Badachi, Shiraguppi and Kerwadi.

4.1.2 October pruning

The survey was carried out during September to November period. The disease intensity was comparatively more in the April pruned vineyards. The disease was severe (grade 4) at Tikota having 31 – 50 per cent infection of grapevine, whereas the disease was moderately severe (grade 3) in Jumanal, Tidagundi, Bihharagi, Chikkapadasalagi, Lokapur, Athani and Shiraguppi having 16 – 30 per cent infection of grapevines. But, disease was moderate or mild (grade 2) in Kerwadi having 6 – 15 per cent infection.

4.2 Symptomatology

The symptoms such as, production of minute water soaked lesions on leaves especially on the veins and veinlets, irregular to angular in shape and cankerous, one to four mm in diameter leading to leaf necrosis. In severe cases, the pathogen also infects inflorescence and tender shoots, cankers lesions on the canes and causing considerable yield loss and quality of the produce. The lesions were dark brown light yellow in colour. But with age enlarge or coalease to form irregular necrotic cankerous patches. Hence, the disease is also named as bacterial canker (Plate 2).

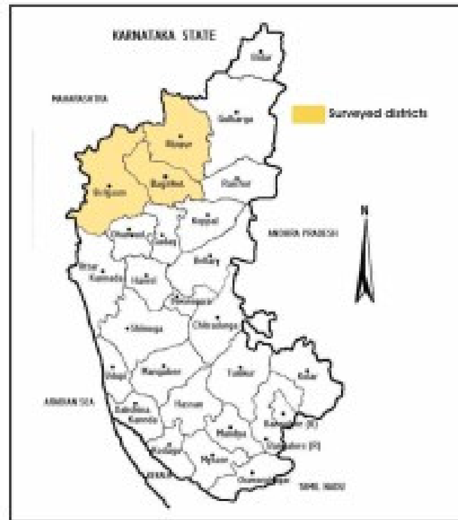
4.3 Isolation of *Xanthomonas campestris* pv. *viticola* from infected grape leaves

The ooze test was conducted by cutting a small bit of the leaf tissue from the infected leaves and suspending it in a drop of sterile water taken on a microscopic glass slide and observing under low power objective of the microscope. Within few minutes the bacterial ooze started jetting out from the cut ends of the infected tissue, thus revealing the association of bacteria with the disease.

Isolation was made from the bacterial ooze obtained from the infected leaf tissue in sterile distilled water followed by dilution plate technique on nutrient agar produced typical *Xanthomonas* colonies in 48 hours. The colonies were yellow, mycoid, slimy, glistening, convex, colourless and round in shape (Plate 3).

Table 2: Survey for bacterial canker of grapes in Northern Karnataka

District	Location	Disease severity (0-5 scale)	
		April pruning	October pruning
Bijapur	Jumanal	4	3
	Tidagundi	3	3
	Tikota	5	4
	Bijjaragi	4	3
Bagalkot	Chikkapadasalagi	5	3
	Lokapur	3	3
Belgaum	Athani	3	3
	Badachi	3	2
	Shiraguppi	3	3
	Kerwadi	3	2



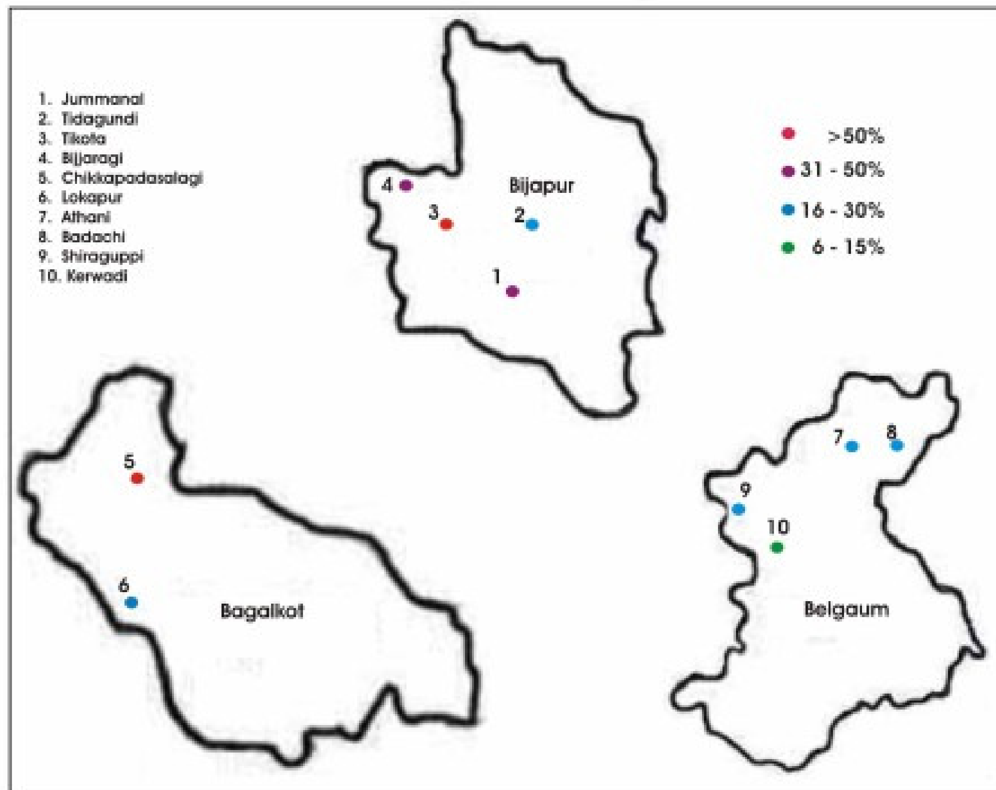


Plate 1: Survey on severity bo bacterial leaf spot of grape in Northern Karnataka during 2007-08

4.3.1 Purification of bacterial culture

Well separated out colonies isolated from the infected leaves were purified by streaking on the surface of YDCA medium. The culture was stored on nutrient agar slants at 5^0C and also suspended few loopful of culture in sterile distilled water contained in ampules. These were kept as the stock cultures for further studies. The bacterial colonies on YDCA medium were deep yellow, slimy, highly viscious and irregular to round in shape.

4.3.2 Identification of causal organism

Morphological, physiologic and biochemical characteristics

The results of the various morphological, physiologic and biochemical tests are given in Table 3 and Plate 4. The isolate was found to be rod shaped, obligately aerobic, gram negative, oxidase negative and monotricously flagellated, but not utilized asparagines as a sole source of carbon and nitrogen. It was positive for catalase reaction utilized glucose, fructose, sucrose for acid production, liquefaction of gelatin and produced hydrogen sulphide and did not produce indole, in addition, the strain failed to reduce nitrate to nitrites (Plate 4).

a. Utilization of glucose, sucrose, fructose for acid production

It was observed that after 72 hours of incubation, there was production of gas in Durhams tubes and colour of the medium changed to yellow (Plate 4).

b. Utilization of asparagines as sole source of carbon and nitrogen

After three days of incubation at 30^0C examined no growth. The bacterium showed negative reaction for this test.

c. Catalase test

After covering with few drops of 20 volume hydrogen peroxide to a slide smeared by a loopful of test bacteria, there was production of gas bubbles.

d. Gelatin liquefaction

In this test, observed that liquefaction of the gel column in the test tube inoculated with test bacterium and no liquefaction of gel when kept as control (Plate 4).

e. Methyl red reaction

After adding a few drops of 0.02 per cent methyl red in 50 per cent alcohol to the culture tube. There was no any distinct red colour. The bacterium showed negative reaction for this test.

f. Reduction of nitrate to nitrite

It was observed that, nitrate was not reduced by this bacterium and colour of the broth become pink.

g. Urease test

In this test, there was no any change of medium colour in the inoculated slants from yellow to red. The bacterium showed negative reaction.

h. Indole production

There was no colour change of the oxalic acid crystals on the test strip.

i. Hydrogen sulphide production

In which, observed that bacterium of test strips indicated liberation of H_2S. The bacterium showed positive reaction for this test (Plate 4).

Leaf infection

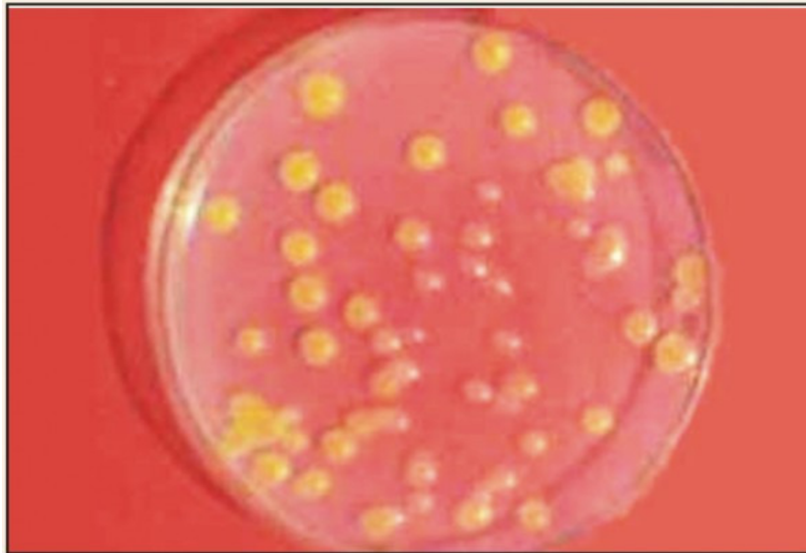
Leaf drying

Cane infection

Petiole infection

Stem infection

Plate 2: Symptomatology of bacterial leaf spot of grapevine



X. c. pv. *viticola* colonies on nutrient agar media

Growth on nutrient broth

Plate 3: Isolation of Xanthomona campestris pv. Viticola on nutrient agar

j. Oxidase test

It was observed that no red or purple colour appeared within 10 seconds.

k. Starch hydrolysis

It was observed that a colourless zone around the bacterial growth in contrast to the blue background of the medium (Plate 4).

4.3.3 Hypersensitive reaction on tobacco

The strain of *X. campestris* pv. *viticola* injected into intercellular spaces of tobacco leaves (*Nicotiana tobaccum* var. *samsun*) produced characteristics water soaked lesions within 16 to 20 hours of inoculation. Further, after 24 hours the light yellow area started collapsing and formed a desiccated light brown necrotic area (Plate 5).

4.3.4 Pathogenicity of grape

The strain of *X. campestris* pv. *viticola* artificially inoculated to 30 days old grape seedlings, cv. Thompson seedless, by spraying the bacterial culture to pre-injured leaves. The plants started producing small water soaked lesions on the leaves. The lesions became necrotic leading to blighting of infected leaves. The first symptoms of the disease observed 15 days after inoculation of the plants (Plate 6).

Re-isolations of the bacterium made from artificially inoculated plants yielded yellow coloured colonies on YDCA and the strain was confirmed as *Xanthomonas campestris* pv. *viticola*.

4.4 *In-vitro* evaluation of different chemicals alone and their combination against the growth of *Xanthomonas campestris* pv. *viticola*

Evaluation of chemicals was done by paper disc method as described in Material and Methods. The results on the efficiency of various chemicals in inhibiting the growth of bacterium expressed as inhibition zone (mm) are presented in Table 4, Fig. 1 and Plate 6.

Among the various chemicals (bactericide/antibiotics/bioagents alone and in their combination) tested, streptocycline 500 ppm + copper oxychloride 2000 ppm produced a highest inhibition zone of 24.97 mm, followed by streptocycline alone at 500 ppm produced 22.40 mm inhibition zone and was on par with agrimycin 500 ppm + copper oxychloride 2000 ppm (21.70 mm), bacteriocare 500 ppm plus copper oxychloride 2000 ppm (21.40 mm)..

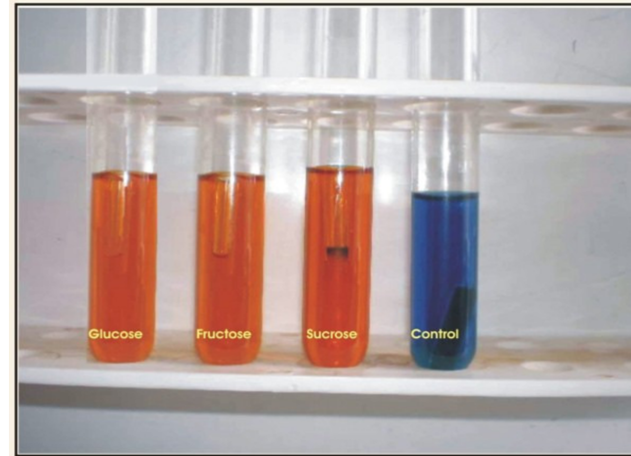
4.4.1 Effect of antagonistic microorganisms on the growth of *X. campestris* pv. *viticola* under *in-vitro* condition

The results are presented in Table 4, Fig. 1 and Plate 6, the antagonistic organism such as *Pseudomonas fluorescens* and *Bacillus subtilis* were tested for their effect on the growth of *Xanthomonas campestris* pv. *viticola*. *Bacillus subtilis* was found to be more effective (8.20 mm) in inhibiting the growth of the pathogen and *Pseudomonas fluorescens* was produced an inhibition zone of (7.00 mm).

Table 3: Morphological and biochemical characteristics of *Xanthomonas campestris* pv. *viticola* causing bacterial leaf spot of grape

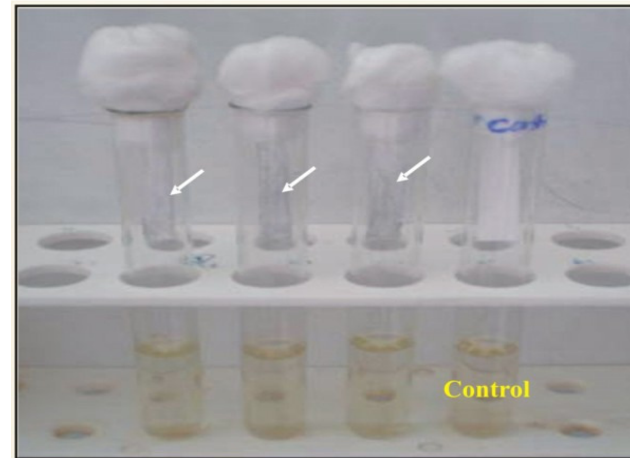
Characters	Isolate
1. Morphology : shape	Small rods
a. Occurrence	In single
b. Flagelation	Monotrichous
II. staining	
a. Gram reaction	G –ve
b. Capsule staining	+
c. Spore staining	-
d. Acid fast	-
III. Biochemical characters	
a. Utilization of glucose, sucrose, fructose for acid production	+
b. Utilization 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	+

Starch hydrolysis



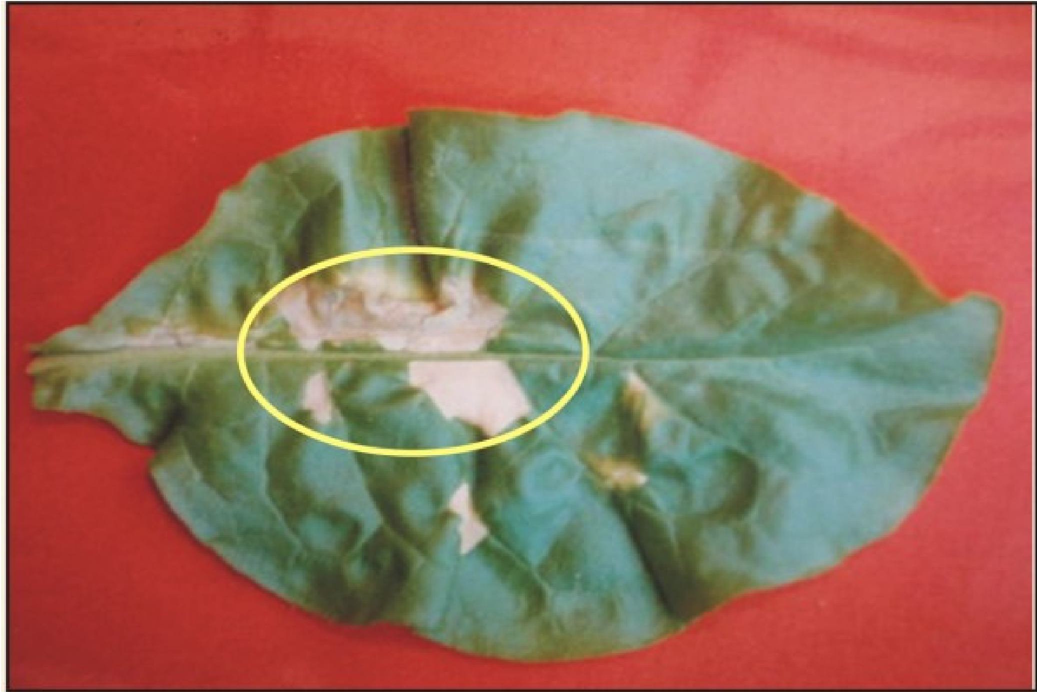
Acid production

Gelatin Liquefaction



Hydrogen sulphide production

Plate 4: Identification of causal organism



Hypersensitive reaction on tobacco

Pathogenicity test on grape plant

Plate 5: Pathogencity studies

Table 4: Effect of chemicals and their combination on the growth of *Xanthomonas campestris* pv. *viticola* under *In-vitro* condition

Sl. No.	Antibiotic / Bactericide	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
	SEm$\pm$		0.51
	CD at 1%		2.18

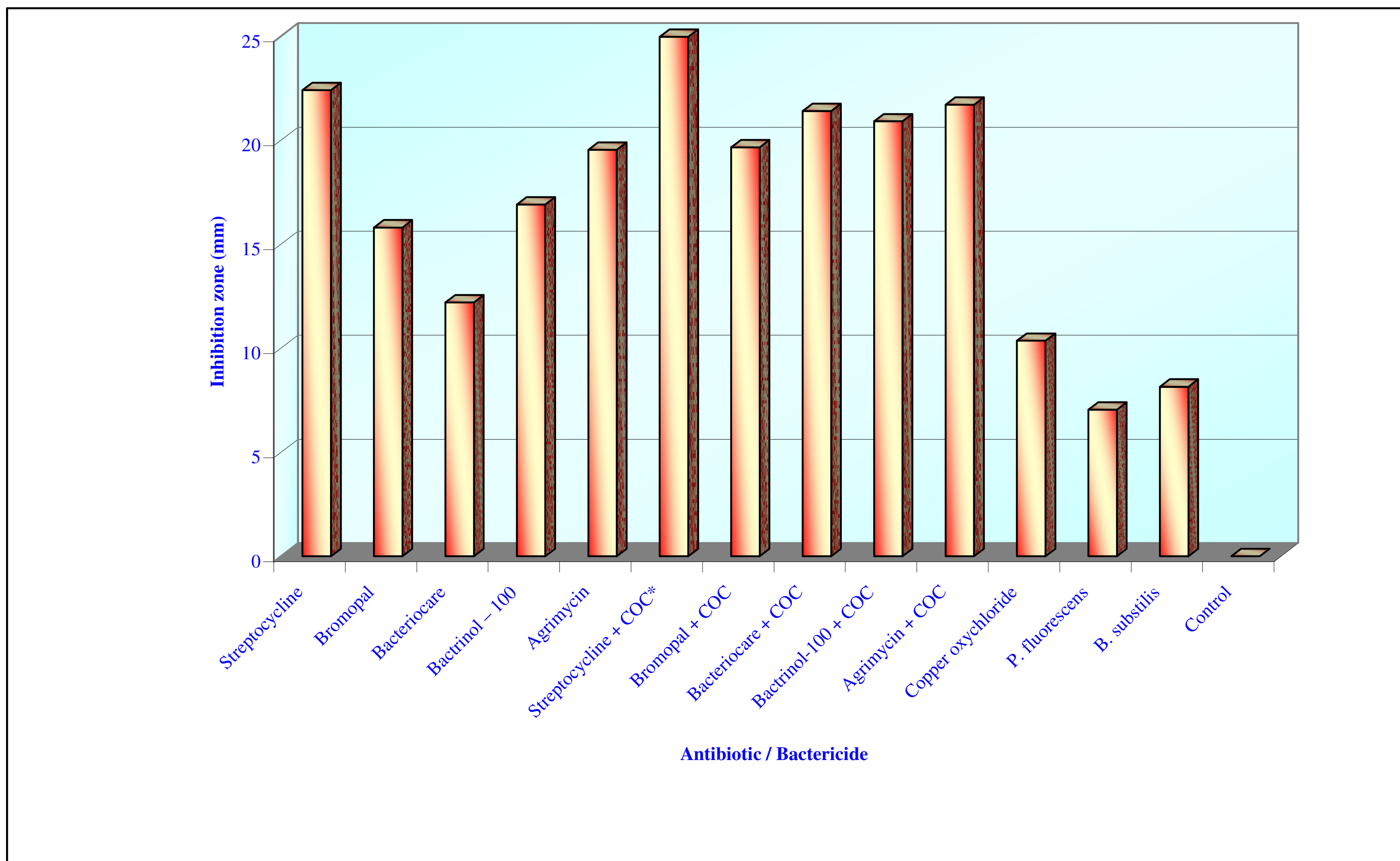
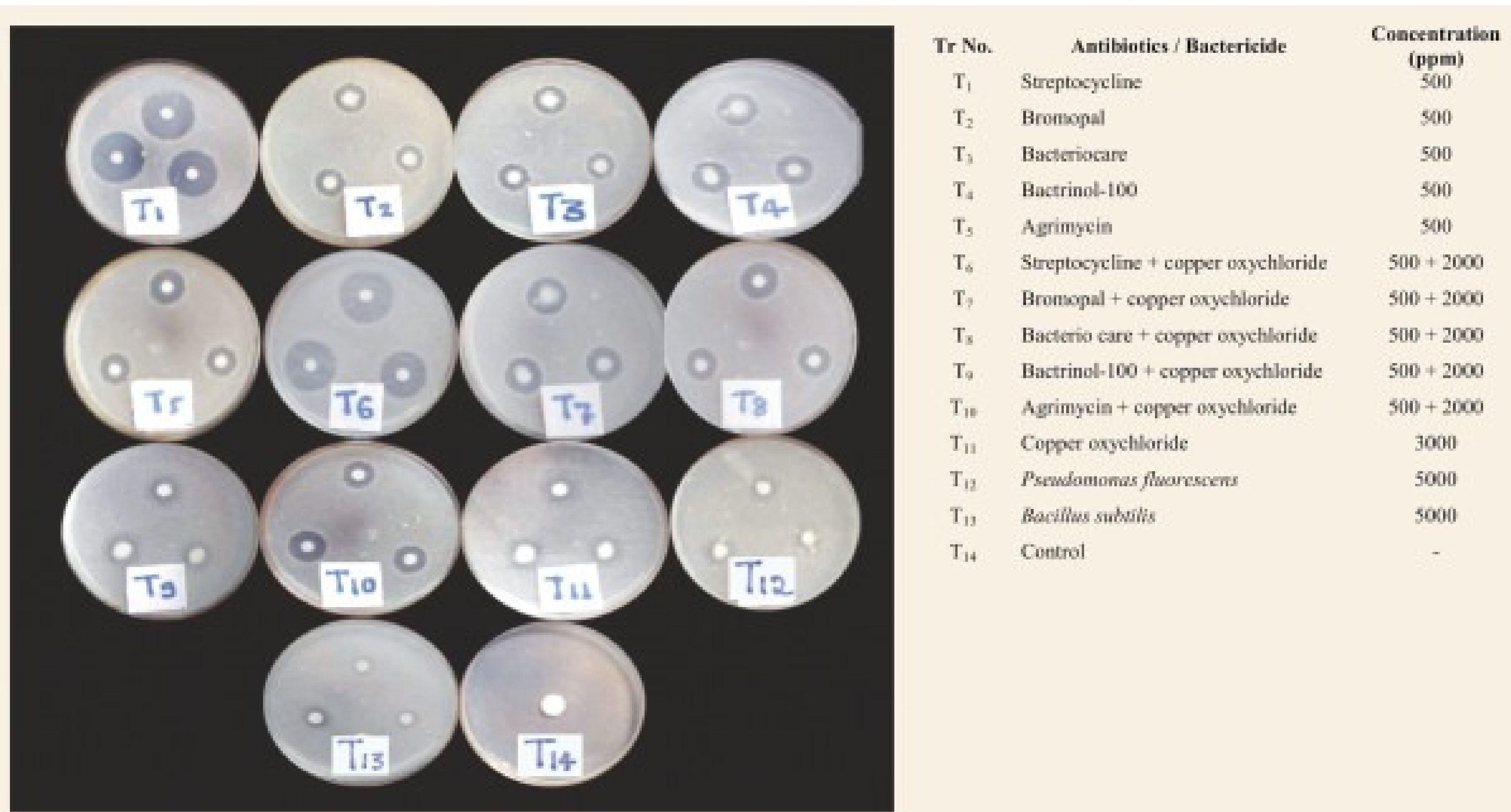


Fig. 1: Effect of different chemicals, bioagents alone and their combination on the growth of Xanthomonas campestris pv. viticola under In-vitro condition



Tr No.	Antibiotics / Bactericide	Concentration (ppm)
T_1	Streptocycline	500
T_2	Bromopal	500
T_3	Bacteriocare	500
T_4	Bactrinol-100	500
T_5	Agrimycin	500
T_6	Streptocycline + copper oxychloride	500 + 2000
T_7	Bromopal + copper oxychloride	500 + 2000
T_8	Bacterio care + copper oxychloride	500 + 2000
T_9	Bactrinol-100 + copper oxychloride	500 + 2000
T_{10}	Agrimycin + copper oxychloride	500 + 2000
T_{11}	Copper oxychloride	3000
T_{12}	*Pseudomonas fluorescens*	5000
T_{13}	*Bacillus subtilis*	5000
T_{14}	Control	-

Plate 6: Effects of chemicals and their combination on the growth of Xanthomonas campestris pv. Viticol

4.5 *In-vivo* evaluation of different chemicals alone and their combination against the growth of *Xanthomonas campestris* pv. *viticola*

4.5.1 Per cent disease index (PDI)

The results obtained are presented in Table 5, Fig. 2 and Plate 7.

The results obtained at the end of the first spray indicated that, streptocycline 500 ppm plus copper oxychloride 2000 ppm was found effective (PDI = 36.20%) in managing the bacterial canker of grapes followed by streptocycline 500 ppm (PDI = 40.50%) and was on par with agrimycin 500 ppm (PDI = 45.18%), bacteriocare 500 ppm plus copper oxychloride 2000 ppm (PDI = 42.10%), agrimycin 500 ppm plus copper oxychloride 2000 ppm (PDI = 41.50%). Maximum incidence was recorded in untreated check. (58.10%).

At the end of second spray it was observed that, streptocycline 500 ppm plus copper oxychloride 2000 ppm was found effective (PDI = 30.95%), followed with streptocycline 500 ppm (PDI = 36.50%) and was on par with bacteriocare + copper oxychloride (37.60%), bromopal 500 ppm + copper oxychloride (PDI = 37.80%), bactrinol 500 ppm + copper oxychloride 2000 ppm (PDI = 39.30%), agrimycin 500 ppm + copper oxychloride 2000 ppm (PDI = 38.83%), bactrinol 500 ppm (42.20%), bromopal 500 ppm (PDI = 38.50%) and PDI = 61.06 per cent in control.

Results obtained at the end of third spray observed that, streptocycline 500 ppm plus copper oxychloride 2000 ppm was found the best (PDI = 22.50%) followed by streptocycline 5000 ppm (PDI = 28.05%) and was on par with bacteriocare 500 ppm (PDI = 32.45%), bacteriocare 500 ppm plus copper oxychloride 2000 ppm (PDI = 31.60%), bacterinol 500 ppm plus copper oxychloride 2000 ppm (PDI = 32.70%). The maximum disease pressure was recorded in control (PDI = 64.80%).

The lowest mean per cent disease index was observed in streptocycline 500 ppm plus copper oxychloride 2000 ppm (mean PDI = 29.86%). This was followed by streptocycline 500 ppm (mean PDI = 35.35%).

It was found that highest per cent control over check was observed in plants sprayed with streptocycline 500 ppm plus copper oxychloride 2000 ppm (47.85%) followed by streptocycline 500 ppm (40.72%) and least was *P. fluorescens* 5000 ppm (23.21%).

4.5.2 Number of leaves infected per leaflet before and after spray

The minimum number of infected leaves per leaflet was observed (Table 6 and Fig. 3) in plants sprayed with streptocycline 500 ppm plus copper oxychloride 2000 ppm (4.35), which was significantly superior over other chemicals. The next best treatments were streptocycline 500 ppm (7.05), agrimycin 500 ppm (9.05), bacteriocare 500 ppm plus copper oxychloride 2000 ppm (12.03 – 8.08), agrimycin 500 ppm plus copper oxychloride 2000 ppm (12.03 – 8.03). The biocontrol agents were not effective in more infected leaves per leaflet *P. fluorescens* 5000 ppm (21.48 – 19.05); *Bacillus subtilis* 5000 ppm gave good result (20.33 – 17.50)). In control, the number of infected leaves was increased from 23.28 – 25.60 (Table 6).

4.5.3 Severity of disease before and after spray

The results are presented in Table 7 and Fig. 4. The highest per cent reduction of disease severity was observed in streptocycline 500 ppm plus copper oxychloride 2000 ppm (49.15% – 28.50%), which was found to be superior to the other treatments followed by streptocycline 500 ppm (45.65% - 31.30%) and was on par with agrimycin 500 ppm + copper oxychloride 2000 ppm (49.90% - 36.10%), bacteriocare 500 ppm + copper oxychloride 2000 ppm (47.60 – 32.20%). Among the bioagents, *Bacillus subtilis* 5000 ppm was found be good (51.30% - 43.32%) when compared to *P. fluorescens* 5000 ppm (52.70% - 47.05%). Untreated control recorded maximum severity range of 50.80 to 62.65 per cent.

Table 5: *In-vivo* evaluation of different chemicals alone and their combination against of *Xanthomonas campestris* pv. *viticola*

Sl. No.	Treatments	Conc. (ppm)	PDI				% control over check
			1st spray	2nd spray	3rd spray	Mean	
1.	Streptocycline	500	40.50 (40.90)*	36.50 (38.93)	28.05 (31.98)	35.35	40.72
2.	Bromopal	500	51.88 (46.07)	38.50 (38.35)	35.50 (36.56)	41.96	31.57
3.	Bacteriocare	500	49.30 (44.59)	44.00 (41.52)	32.45 (34.54)	41.82	31.81
4.	Bactrinol – 100	500	48.05 (43.88)	42.20 (40.48)	33.40 (35.30)	41.22	32.79
5.	Agrimycin	500	45.18 (42.22)	41.10 (39.84)	36.30 (37.04)	40.86	33.37
6.	Streptocycline + COC*	500+2000	36.20 (36.92)	30.95 (35.61)	22.45 (27.97)	29.86	47.85
7.	Bromopal + COC	500+2000	50.20 (45.11)	37.80 (37.93)	36.40 (37.08)	41.47	32.38
8.	Bacteriocare + COC	500+2000	42.10 (40.42)	37.60 (37.81)	31.60 (34.19)	37.10	39.50
9.	Bactrino-100l + COC	500+2000	46.75 (43.13)	39.30 (38.80)	32.70 (34.82)	39.58	35.45
10.	Agrimycin + COC	500+2000	41.50 (39.51)	38.83 (38.35)	33.20 (35.17)	37.41	39.00
11.	Copper oxychloride	3000	48.55 (44.16)	43.95 (41.52)	42.20 (40.51)	44.90	26.78
12.	*P. fluorescens*	5000	51.85 (46.05)	45.04 (41.15)	44.38 (41.76)	47.09	23.21
13.	*B. subtilis*	5000	48.25 (43.09)	44.50 (41.83)	43.70 (41.36)	45.48	25.83
14.	Control	-	58.10 (99.66)	61.06 (51.39)	64.80 (53.61)	61.32	-
	SEm$\pm$		1.80	1.87	1.89		
	CD at 5%		5.49	5.72	5.77		

*COC – Copper oxychloride
*Figures in parentheses indicate arc sine values

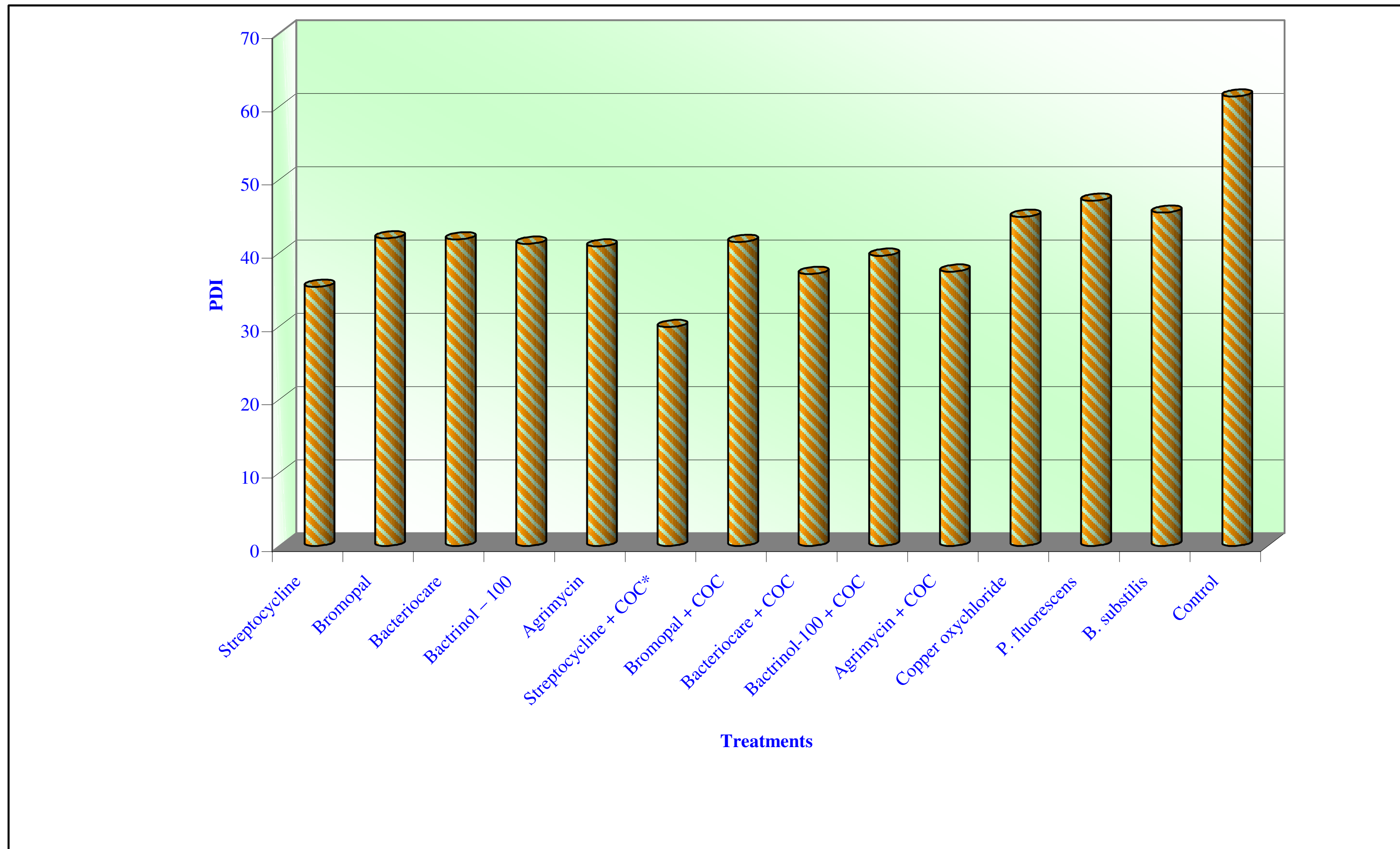


Fig. 2: In-vivo evaluation of different chemicals alone and their combination against of Xanthomonas campestris pv. viticola

Plate 7: In-vitro evaluation of different chemicals alone and their combination against the growth of *Xanthomonas campestris* pv. *viticola*

Table 6: Number of leaves infected per leaflet before and after spray

Sl. No.	Treatments	Conc. (ppm)	Mean No. of leaves infected /leaflet	
			Before spray	After spray
1.	Streptocycline	500	12.03	7.05
2.	Bromopal	500	16.10	11.10
3.	Bacteriocare	500	12.25	10.08
4.	Bactrinol – 100	500	16.05	13.35
5.	Agrimycin	500	12.10	9.05
6.	Streptocycline + COC*	500+2000	13.23	4.15
7.	Bromopal + COC	500+2000	15.18	11.13
8.	Bacteriocare + COC	500+2000	12.03	8.08
9.	Bactrinol-100 + COC	500+2000	16.20	12.05
10.	Agrimycin + COC	500+2000	12.03	8.03
11.	Copper oxychloride	3000	18.30	15.10
12.	*P. fluorescens*	5000	21.48	19.05
13.	*B. subtilis*	5000	20.23	17.50
14.	Control	-	23.28	25.60
	SEm±		1.05	0.92
	CD at 5%		3.20	2.80

*COC – Copper oxychloride

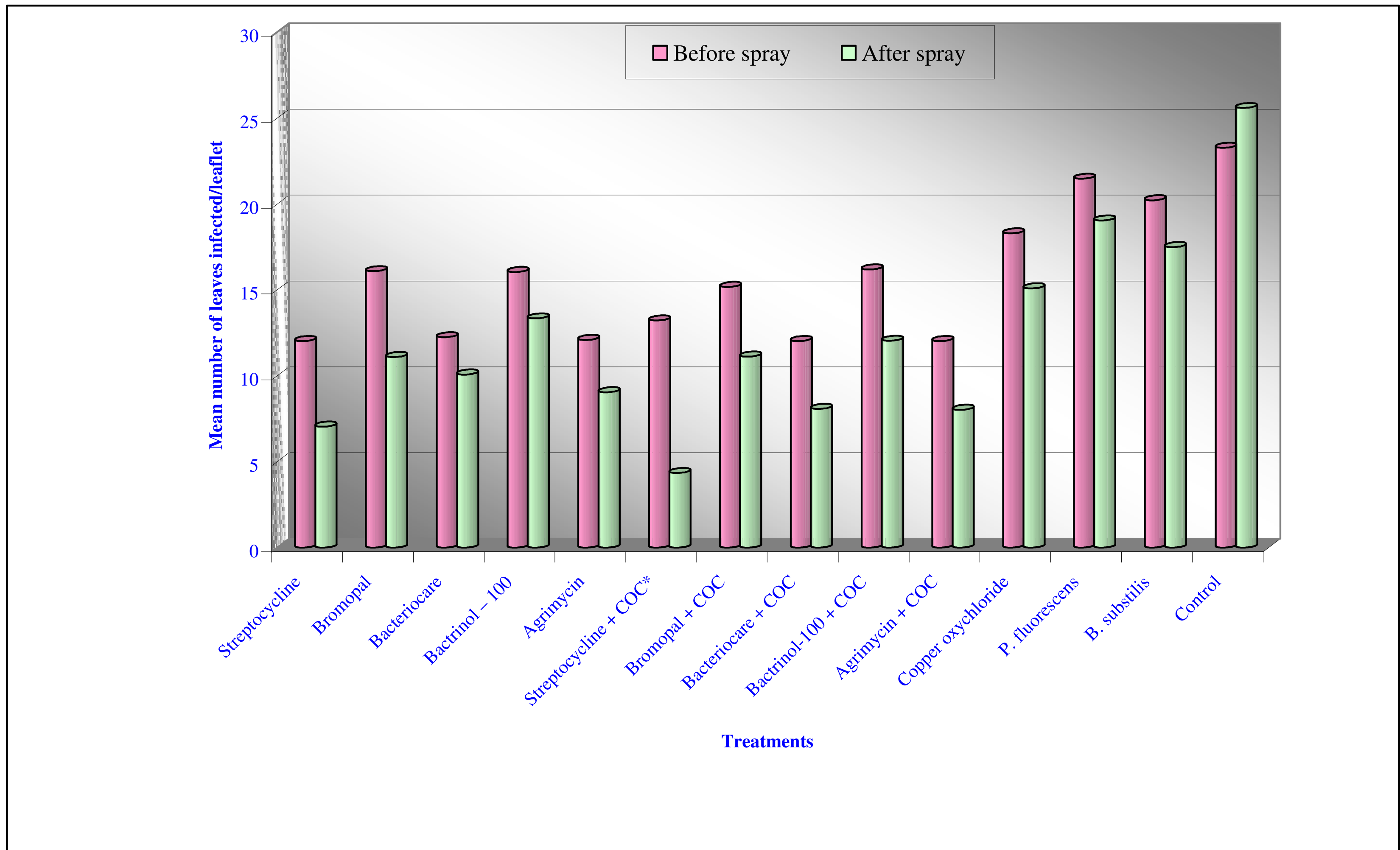


Fig. 3: Number of leaves infected per leaflet before and after spray

Table 7: Severity of disease before and after spray

Sl. No.	Treatments	Conc. (ppm)	Severity	
			Before spray	After spray
1.	Streptocycline	500	45.65 (42.50)*	31.30 (34.62)
2.	Bromopal	500	52.50 (46.43)	41.20 (39.92)
3.	Bacteriocare	500	51.00 (45.57)	37.80 (37.93)
4.	Bactrinol – 100	500	52.30 (46.31)	40.70 (39.63)
5.	Agrimycin	500	48.20 (43.96)	36.90 (37.40)
6.	Streptocycline + COC*	500+2000	49.15 (44.51)	28.50 (32.23)
7.	Bromopal + COC	500+2000	54.10 (47.35)	40.50 (39.52)
8.	Bacteriocare + COC	500+2000	47.60 (43.62)	32.20 (35.16)
9.	Bactrinol-100 + COC	500+2000	49.65 (44.79)	37.20 (37.58)
10.	Agrimycin + COC	500+2000	49.90 (44.94)	36.10 (36.92)
11.	Copper oxychloride	3000	49.25 (44.56)	40.30 (39.40)
12.	*P. fluorescens*	5000	52.70 (46.54)	47.05 (43.29)
13.	*B. subtilis*	5000	51.30 (45.74)	43.32 (41.13)
14.	Control	-	50.80 (45.46)	62.65 (52.32)
	SEm±		1.64	1.60
	CD at 5%		5.01	4.88

*COC – Copper oxychloride
*Figures in parentheses indicate arc sine values

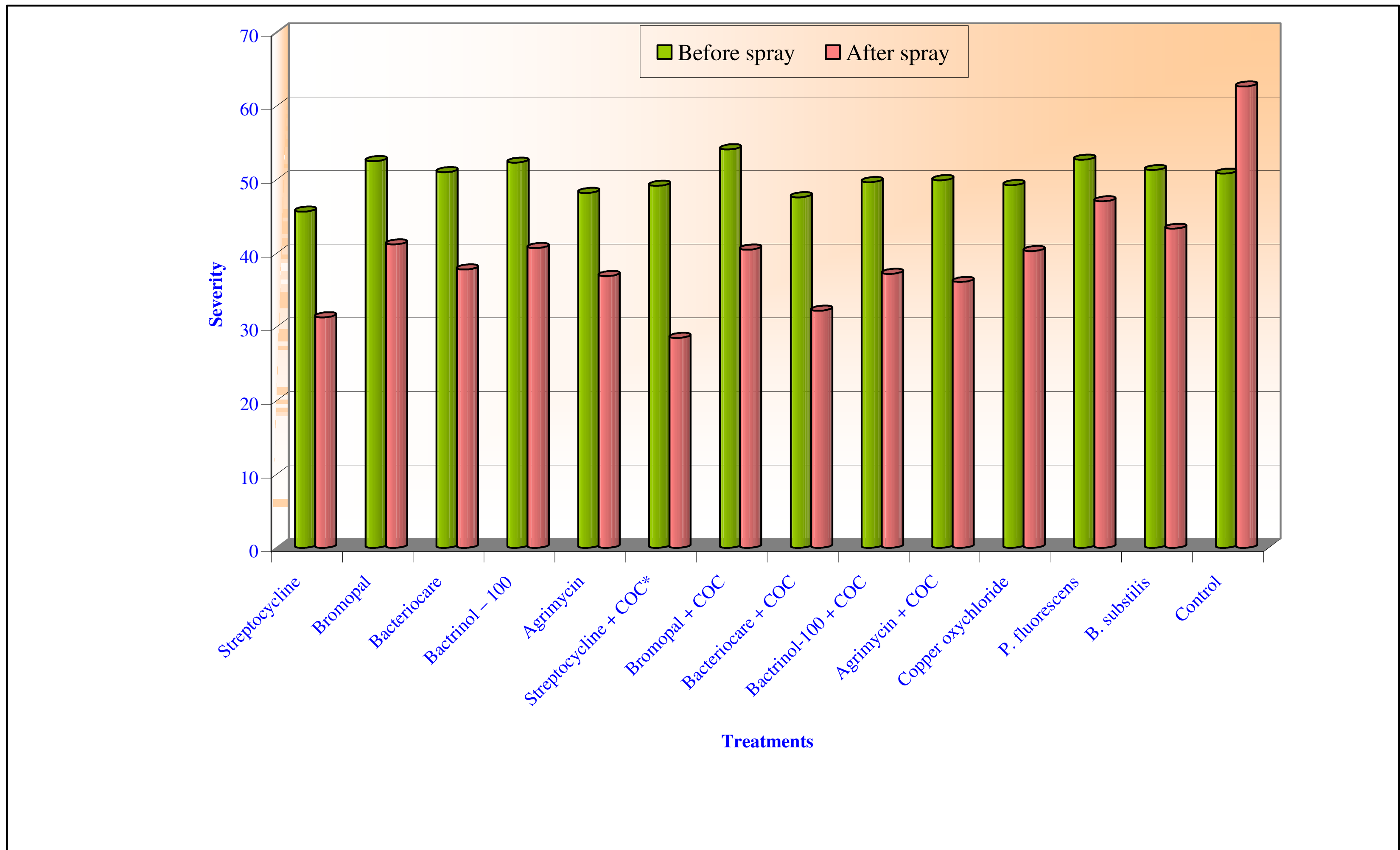


Fig. 4: Severity of disease before and after spray

Table 8: Yield and yield parameters

Sl. No.	Treatments	Conc. (ppm)	Yield (t/ha)	Mean No. of bunches production/plant	Mean No. of bunches infected/plant	Mean weight of single bunch (g)
1.	Streptocycline	500	23.50	17.13	5.05	861
2.	Bromopal	500	17.13	12.23	7.07	684
3.	Bacteriocare	500	19.25	14.15	6.40	760
4.	Bactrinol – 100	500	21.30	13.10	8.35	817
5.	Agrimycin	500	21.85	15.05	7.03	835
6.	Streptocycline + COC*	500+2000	26.95	20.03	3.40	925
7.	Bromopal + COC	500+2000	18.33	13.75	6.15	716
8.	Bacteriocare + COC	500+2000	20.18	15.20	5.25	785
9.	Bactrinol-100 + COC	500+2000	22.53	14.48	7.01	844
10.	Agrimycin + COC	500+2000	23.35	16.63	6.05	856
11.	Copper oxychloride	3000	16.73	12.42	9.09	619
12.	*P. fluorescens*	5000	11.35	7.43	7.08	318
13.	*B. subtilis*	5000	14.23	9.35	7.10	413
14.	Control	-	9.80	6.17	6.05	273
	SEm±		1.53	0.89	0.43	0.05
	CD at 5%		4.68	2.73	1.31	0.15

*COC – Copper oxychloride

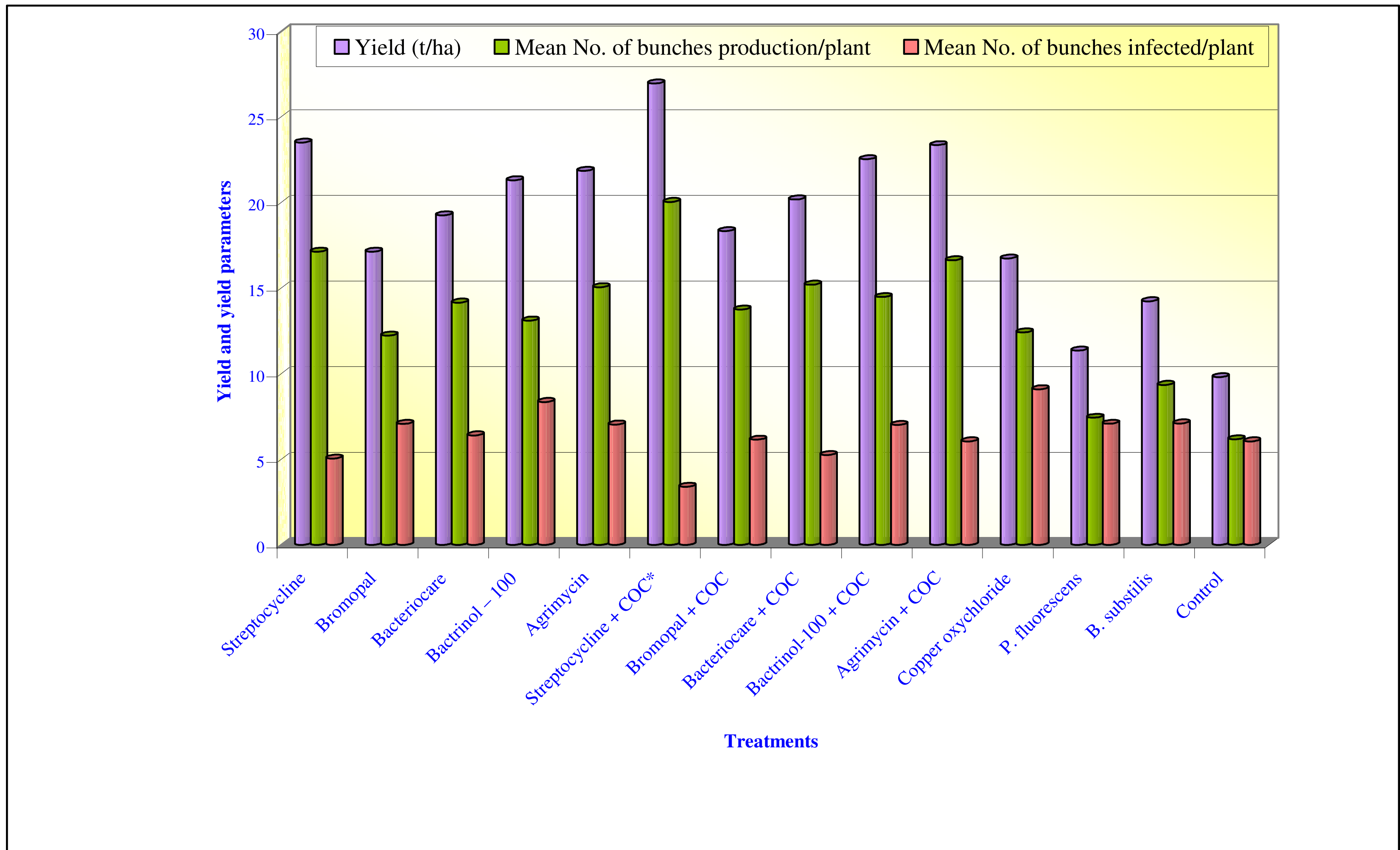


Fig. 5: Yield and yield parameters

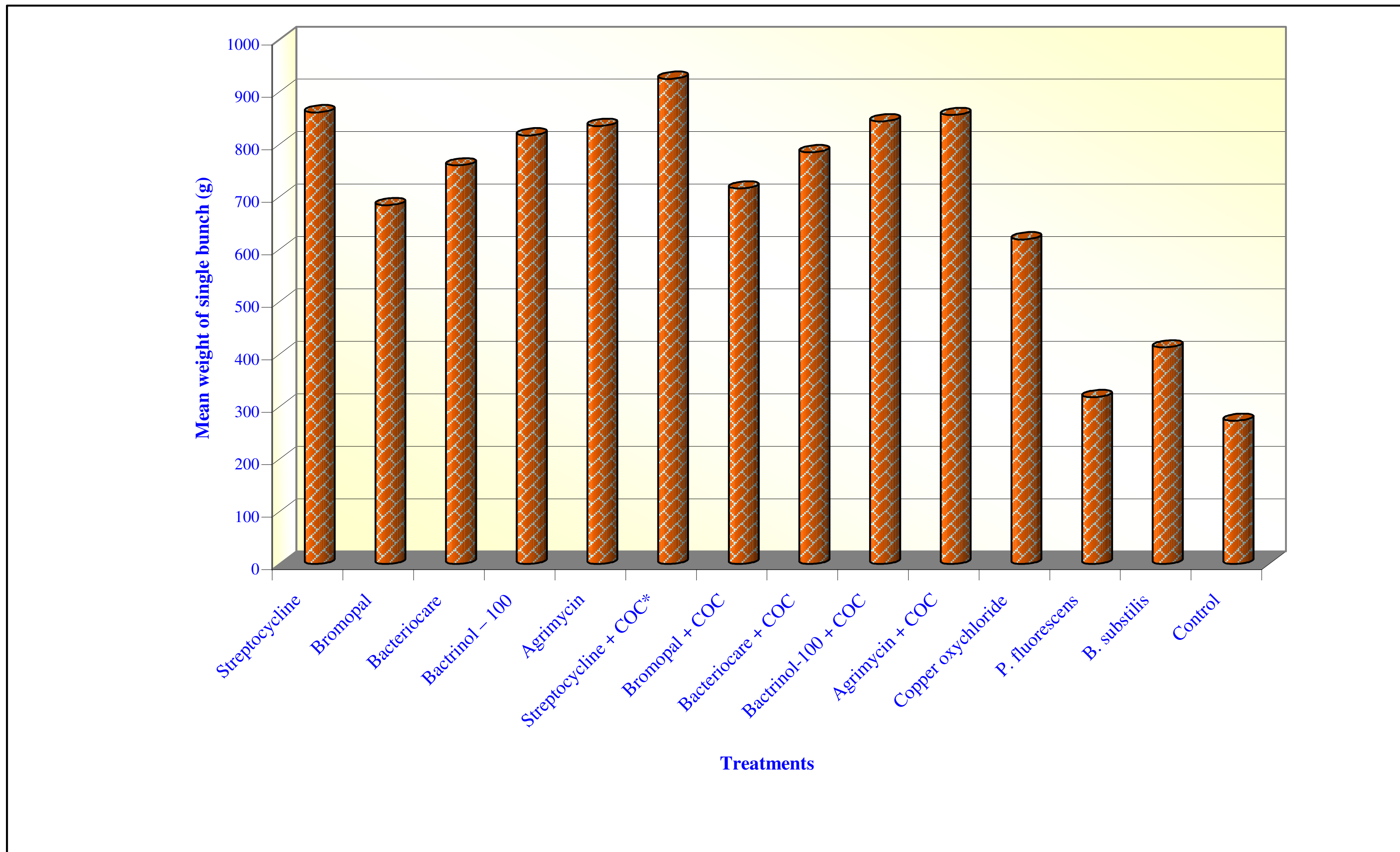


Fig. 6: Yield and yield parameters

Table 9: Economic evaluation of bactericides, antibiotics and bioagents alone and their combination against *Xanthomonas compestris* pv. *viticola* under field condition

Sl. No.	Treatments	Conc. (ppm)	Yield (t/ha)	Gross returns (Rs./ha)	Cost of cultivation (Rs./ha)	Net returns (Rs./ha)	B:C ratio
1.	Streptocycline	500	23.50	352500	108340	244160	2.25
2.	Bromopal	500	17.13	256950	107542	149408	1.38
3.	Bacteriocare	500	19.25	288750	108540	180210	1.66
4.	Bactrinol – 100	500	21.30	319500	107542	211958	1.97
5.	Agrimycin	500	21.85	327750	108340	219410	2.02
6.	Streptocycline + COC*	500+2000	26.95	404250	111040	293210	2.64
7.	Bromopal + COC	500+2000	18.33	274950	110242	164708	1.49
8.	Bacteriocare + COC	500+2000	20.18	302700	111240	191460	1.72
9.	Bactrinol-100 + COC	500+2000	22.53	337950	110242	227708	2.06
10.	Agrimycin + COC	500+2000	23.35	350250	111040	239210	2.15
11.	Copper oxychloride	3000	16.73	250950	109092	240048	2.20
12.	*P. fluorescens*	5000	11.35	170250	105642	64608	0.61
13.	*B. subtilis*	5000	14.23	213450	105642	107808	1.02
14.	Control	-	9.80	104800	-	-	-

4.5.4 Yield and yield parameters

The data on yield and yield parameters are presented in Table 8 and Fig. 5 and 6. The results revealed that the highest fruit yield of (26.95 t/ha) was observed in streptocycline 500 ppm plus copper oxychloride 2000 ppm closely followed by streptocycline 500 ppm (23.50 t/ha) and were on par with each other. The least yield was obtained in control (9.08 t/ha). *Bacillus subtilis* 5000 ppm gave better yield (14.23 t/ha) than *P. fluorescens* 5000 ppm (11.35 t/ha).

The more number of bunches production was observed in plants sprayed with streptocycline 500 ppm plus copper oxychloride 2000 ppm (20.03) followed by streptocycline 500 ppm (17.13) and was on par with each other. Least number of bunches production was observed in control (6.17) and was on par with *Pseudomonas fluorescens* 5000 ppm (7.43).

The lowest number of bunches infected was observed in plants sprayed with streptocycline 500 ppm plus copper oxychloride 2000 ppm (3.40) followed by streptocycline 500 ppm (5.05) and was on par with bromopal 500 ppm plus copper oxychloride 2000 ppm (6.15), agrimycin 500 ppm plus copper oxychloride 2000 ppm (6.05), bacteriocare 500 ppm plus copper oxychloride 2000 ppm (6.05).

The maximum weight (g) of single bunch was observed in streptocycline 500 ppm plus copper oxychloride 2000 ppm (925 g) followed by streptocycline 500 ppm (862 g) and least weight of a bunch was observed in control (273 g).

5. DISCUSSION

World over from ancient times, grapevine (*Vitis vinifera* L.) has been cultivated mostly for wine making. On the contrary in India, remarkable success has been achieved in table grape production and the yield levels of fresh grapes are among the highest in the world. Grape has gained global economic significance due to good back of grape industry in terms of backward and forward linkage that offers employment to large number of skilled and unskilled people on the farm, trade and service. India has achieved the highest productivity of 20 t per ha in the production of table grapes (Anon., 2007). Despite this rosy scenario, grape cultivation races some such constraints as SPS (Sanitary and Phytosanitary) issues including pests and diseases, pest risk analysis (PRA), pesticide residues, which put limitations to the Indian grapes to compete in the global market.

Vines are always vulnerable to fungal and bacterial diseases. Among them downy mildew, powdery mildew, anthracnose and bacterial cankers pose a greater challenge to the grape industry and grape growers in particular.

The bacterial leaf spot of grapes caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye has attained major economic significance in grape growing areas of Northern Karnataka. Bacterial canker of grape was noticed for the first time on *Vitis vinifera* cv. Anab-e-shahi at Tirupati (Andhra Pradesh) during 1960 (Nayudu, 1972). Importance of disease was first visualized in 1983 when it caused substantial loss in yield. During 1986-87, it appeared in the epidemic form in the September pruned vineyards and yield loss was estimated approximately 60 to 80 per cent (Chand and Kishun, 1990a). Presently, this disease has become a regular problem in the early pruned (September) vineyards in the areas of Maharashtra, North Karnataka and Andhra Pradesh.

Taking magnitude of bacterial canker of grape and its resultant losses into consideration the present investigations were undertaken with the different objectives discussed as under.

5.1 Survey for bacterial leaf spot of grapes caused *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye in Northern Karnataka

Survey on the severity of the disease both during April and October pruning stages revealed the magnitude of the problem on hand, served as basis to identify or evolve the strategies in the management of disease. Hence, present investigation survey was carried out in northern parts of Karnataka covering Bijapur, Bagalkot and Belgaum districts. It was evident that the bacterial canker of grape was more severe during April pruned stages of crop than October pruned stage. The maximum severity of disease (grade 5) in April pruned crop was recorded in Tikota and Chikkapadasalagi having more than 50 per cent of infection of grapevine. Due to rainfall in combination with optimum temperature (25 – 30^{0}C) with moderate wind lead to severity of disease (Chand and Kishun, 1990b) and the relative humidity above 80 per cent indirectly helps to more inoculum build up leads to disease epidemic (Chand, 1996). Whereas, the disease was severe (grade 4) in Jumanal and Bijjaragi having 31 to 50 per cent infection of grapevine and disease was moderately severe (grade 3) at Tidagundi, Lokapur, Badachi, Shiraguppi and Kerwadi. During this period, severity more in orchards pruned before 15th of September. Similar result was obtained by Chand and Patil (1993).

In October pruned grapevine, the disease was severe (grade 4) at Tikota having 31 to 50 per cent infection of grapevines, whereas disease was moderately severe (grade 3) in Jumanal, Tidagundi, Bijjaragi, Chikkapadasalagi, Lokapur, Athani and Shiraguppi having 16 to 30 per cent infection of grapevines. But, in disease was moderate or mild (grade 2) in Kerwadi having 6 to 15 per cent infection.

5.2 Symptomatology

The typical symptoms of bacterial cankers of grapes caused by *X. campestris* pv. *viticola* are production of water soaked lesions on leaves, irregular to angular in shape and cankerous, 1-4 mm in diameter, especially on veins and veinlets leading to leaf necrosis. In severe cases, the pathogen also infected inflourescence and tender shoots, cankerous lesions on the canes, the lesions were dark brown in colour. But, with age enlarged or coaleased to form irregular necrotic patches. Hence, of the disease as bacterial canker.

Chand and Kishun (1990a) described the symptom of bacterial canker of grapes as on the leaf blade, petiole and cane were angular raised and cankerous lesions under severe infection lead to the death of leaves and affected the growth of canes.

Chand and Kishun (1990b) described the symptoms as in advanced stages of infection, stunting, cracking and irregular growth of canes were also observed. On the berries lesions are brown to black, cankerous and severely infected berries are small and shriveled. Similar symptoms have been recorded by various researchers (Malavolta *et al.*, 1999 and Arauju and Robbs, 2000).

5.3 Isolation, identification and pathogenicity studies

5.3.1 Isolation

The strain *Xanthomonas campestris* pv. *viticola* isolated from infected grape leaves showing typical symptoms of bacterial spot, collected from major grape growing areas of Northern Karnataka. After repeated isolations by the dilution plate method, the infected grape leaves yielded yellow coloured mycoid, slimy, glistening, convex and round colonies. The similar results were obtained by Hayward (1983), Mariano and Gama (2005) and Viana (2006).

5.3.2 Identification

The yellow colonies were identified as *Xanthomonas campestris* pv. *viticola* on the basis of colony and morphological characters, staining reaction, physiologic and biochemical characteristics as per the methods described by Bardbury (1970) and Schaad *et al.* (1988) and hypersensitive reaction on tobacco leaf var. Samsun and on the basis of pathogenicity test on grape plants.

The bacterial cells were small, rod shaped with rounded ends, monotrichouslly flagellated, gram negative and 0.1 to 1.2 $\times$ 2.3 μm in size. On SX agar produced a clear starch digestion zone (3 – 4 min) described by Schaad and White (1974), non-spore forming and non-acid fast. The different physiologic and biochemical characteristics such as utilization of glucose, fructose, success for acid production. The isolate hydrolyzed starch gelatin liquefaction, hydrolyzed casein and produced hydrogen sulphide, the strain did not utilize asparagine as sole source of carbon and nitrogen, positive to catalase reaction, did not produce indole and negative to urease and methyl red reaction, did not reduce nitrate to nitrite. These studies confirmed the identify of pathogen.

5.3.3 Hypersensitive reaction on tobacco

The bacterial isolate produce typical hypersensitive reaction on tobacco (*Nicotiana tobaccum* var. *samsun*) by producing chlorotic spots within 24 hours of inoculation and also the pathogenicity was proved on grape plants.

Thus, on the basis of the pathogenicity test the bacterial isolate was identitical to the pv. *viticola* and on the basis of morphological, physiologic, biochemical and pathogenic studies of the bacterium was designated as *Xanthomonas campestris* pv. *viticola* as described by earlier workers (Bardbury, 1970 and Schaad and Stall, 1988).

5.4 *In-vitro* evaluation of different chemicals alone and their combination against growth of *Xanthomonas campestris* pv. *viticola*

Among the various chemicals tested at different concentrations (bactericides, antibiotics, bioagents, alone and their combination) for their efficacy against inhibiting the growth of *X. campestris* pv. *viticola*. The maximum inhibition zone was observed in streptocycline 500 ppm plus COC which found to be significantly superior over all the other chemicals and bioagents tested. This was previously reported by Chauhan and vaishnav (1980), Sharma and Thind (1981) and Ramesh Chand *et al.* (1991). The next best was streptocyclin. Similar results were reported by many research workers like Chauhan and Vaishnav (1980), Sharma *et al.* (1982) and Namasivayam (1969). The least inhibition zone appeared in *P. fluorescens*.

5.4.1 Effect of antagionistic organisms on the growth of *Xanthomonas campestris* pv. *viticola*

Investigations on the inhibitory effect of antagonistic microorganisms revealed that *Bacillus subtilis* was highly inhibitory followed by *Pseudomonas fluorescens*.

Bacillus subtilis was found to be inhibitory to many of plant pathogenic bacteria (Chen *et al.*, 1990). It was found to be highly antagonistic to *Xanthomonas campestris* pv. *oryzae*. Saktivel *et al.* (1986), Sivamani *et al.* (1989) and Gallardo *et al.* (1989) reported that *Pseudomonas fluorescens* was also inhibitory against wide range of bacteria. Karuna (1993) also noticed that both *Pseudomonas fluorescens* and *Bacillus subtilis* were effective against *R. solanacearum* causing bacterial wilt of tomato under *in-vitro* and *in-vivo* conditions.

5.5 *In-vivo* evaluation of different chemicals alone and their combination against growth of *Xanthomonas campestris* pv. *viticola*

The various chemicals like bactericides, antibiotics, bioagents alone and their combinations at different concentrations were evaluated under field condition. Three sprays were given at 20 day interval, starting from disease development

5.5.1 Per cent disease index (PDI)

Experimental results revealed that streptocycline 500 ppm plus copper oxychloride 2000 ppm was found effective in minimizing the disease incidence. Similar results were obtained by Thirumurthi and Agarwal (1992) and Thombre *et al.* (1989). This was followed by streptocycline 500 ppm, same inference was found by (Gupta, 1977).

The per cent disease control over check was also calculated, highest per cent control over check was observed in streptocycline 500 ppm plus copper oxychloride 2000 ppm, followed by streptocycline 500 ppm.

5.5.2 Number of leaves infected per leaflet before and after spray

The maximum number of infected leaves were reduced in streptocycline 500 ppm plus copper oxychloride 2000 ppm which was found superior over all the other chemicals tested and this was followed by streptocycline 500 ppm. Similar results were enumerated by Ravikumar (1996).

5.5.3 Severity of disease before and after spray

The highest per cent of disease severity was controlled by streptocycline 500 ppm plus copper oxychloride. This combiproduct was found to be effective over all other chemicals followed by streptocycline 500 ppm.

5.5.4 Yield and yield parameters

The yield obtained from streptocycline 500 ppm plus copper oxychloride 2000 ppm was found superior over all other chemicals tested. This was followed by streptocycline alone at 500 ppm. Similar result was found by Raju *et al.* (1980).

The highest number of bunch production, less number of bunches infected per plant and maximum weight of single bunch was observed in streptocycline 500 ppm plus copper oxychloride 2000 ppm and next best was found streptocycline 500 ppm.

Future line of work

1. Screening of disease resistant varieties
2. To study the survival nature of pathogen both in soil and on the host.
3. To study the weather factors in relation with disease incidence.
4. To study on antagonistic bacteria, their interaction with each other and with host plants.
5. Use of seriological techniques, dot immuno-binding assay, ELISA and monoclonal antibodies, PCR methods are essential for the quick detection of disease and differentiation of bacterial strains.

6. SUMMARY AND CONCLUSIONS

Grape is an important temperate fruit crop of the world. It is also cultivated in tropical and sub-tropical regions of the world. In India, especially Northern part of Karnataka produced high quality fresh and table grapes (78%) followed by raisin (17.20%). The raisin production is much concentrated in Bijapur district.

Considerable loss in yield and quality of the produce of the crop has been reported by several workers that deterioration by certain diseases. The bacterial leaf spot of grape is one of the important diseases caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye was noticed for the first time on *Vitis vinifera* pv. Anab-e-shahi at Tirupati (Andhra Pradesh) during 1960. It has attained major economic significance in grape growing areas of Northern Karnataka. The disease has caused extensive damage to yield and quality of produce. In view of destructive nature of bacterial canker of grapes, the present investigations were carried out with the following objectives. To survey for bacterial leaf spot of grapes caused by *Xanthomonas campestris* pv. *viticola* in Northern Karnataka. Isolate, identify and pathogenicity studies. To screen available antibiotics and chemicals against *Xanthomonas campestris* pv. *viticola* under laboratory conditions and integrated management of bacterial leaf spot of grape in field condition.

The fixed plot survey was taken up during 2007-08 in Bijapur, Bagalkot and Belgaum districts of Northern Karnataka both during April and October pruning stages revealed that the highest per cent disease severity was noticed in Tikota village of Bijapur districts, while the least was recorded in Kerwadi of Belgaum district during October pruning stage. Whereas, in April pruning stage, highest per cent disease severity was observed in Tikota and Chikkapadasalagi of Bijapur and Bagalkot districts, respectively and lowest was noticed in Tidagundi village of Bijapur district and in Athani, Shiraguppi, Badachi of Belgaum district.

- Symptoms as producing minute water soaked lesions on leaves initially, especially on veins and veinlets later become light brown in colour leading to leaf necrosis. In severe cases, the pathogen also infects inflorescence and tender shoots, cankerous lesions on canes.

- The bacterium isolated from infected leaves showing typical symptoms of bacterial spot, collected from major grape growing areas of Northern Karnataka, yielded yellow, mycoid, shiny, slimy, convex and odorless colonies on nutrient agar and yeast extract dextrose calcium carbonate agar.

- Based on the physiologic, biochemical and morphological characteristics, the bacterium was identified as *Xanthomonas campestris* pv. *viticola*.

- Further, based on the hypersensitive reaction on *Nicotiana tobaccum* var. *samsun* and pathogenicity tests on grape plants, the bacterium was identified as *Xanthomonas campestris* pv. *viticola.*

- The various bactericides, antibiotics, bioagents alone and their combinations were tested under *in-vitro*, the streptocycline 500 ppm plus copper oxychloride 2000 ppm produced maximum inhibition zone against growth of *Xanthomonas campestris* pv. *viticola* and streptocycline 500 ppm was next in order.

- Among the two bioagents tested against *Xanthomonas campestris* pv. *viticola* under laboratory condition, *Bacillus subtilis* 5000 ppm recorded highest inhibition of radial growth. *Pseudomonas fluorescens* 5000 ppm was found next best.

- In case of field evaluation of bactericides, antibiotics, bioagents. Streptocycline 500 ppm plus copper oxychloride 2000 ppm effectively controlled the disease incidence and recorded minimum per cent disease index followed by streptocycline 500 ppm.

- Among the bioagents, *Bacillus subtilis* 5000 ppm gave comparatively good result than *Pseudomonas fluorescens* 5000 ppm under *in-vivo* condition.

- The yield and yield parameters like number of bunches production and number of bunches infected per plant, weight of single bunch were significantly superior in case of streptocycline 500 ppm plus copper oxychloride 2000 ppm followed by streptocycline 500 ppm.

REFERENCES

Adsule, P. G., Karibasappa, G. S., Banerjee, K. and Mundankar, K., 2007, Status and prospects of raisin industry in India. Paper presented at the *Int. Symp. on Grape Production and Processing*, at Baramati, Maharashtra (India), held on February 6-11, 2006.

Akhtar, M. A. and Aslam, M., 1988, Comparison of methods of inoculation *Xanthomonas campestris* pv. *translucens* in wheat cultivars. *Pakistan J. Bot.,* **20** : 109-112.

*Alcaraz, L. F. De, 1982, Control of citrus canker (*X. citri*) on grape fruits. *Fito Pathol.,* **17** : 48-53.

Anonymous, 1990, *Personal Communication Regarding Bacterinol-100* from Merlin Laboratories, Madras.

Anonymous, 2004, Grape cultivation – Present and future – An overview. *Pestology*, **28** : 13-20.

Anonymous, 2007, *Proc. Int. Symp. on Grape Production and Processing*, at Baramati, Maharashtra (India), held on February 6-11, 2006.

Araujo, J. S. P. and Robbs, C. F., 2000, Symptomatology, pathogenicity and control of bacterial canker of grapevine (*Xanthomonas campestris* pv. *viticola*) in Brazil. *Agronomia*, **34**(1/2) : 83-86.

Ashrafuzzaman, H., 1987, Chemical control of bacterial leaf blight of paddy caused by *Xanthomonas oryzae*. *Plant Pathogenic Bacteria, Mortinus,* Nijhoff Publishers, pp. 955-958.

Balaraman, K., 1987, A new bactericide for citrus canker control. *The Hindu*, August, 19, 1987.

Balaraman, K. and Purushothman, R., 1981, Control of citrus canker on acid lime. *South Indian Hort.*, **29**(4) : 175-177.

Bandyopadhyay, S. and Chattopadhyay, S. B., 1985, Incidence of black rot of cabbage and cauliflower under different condition of infection. *Indian J. Agric. Sci.*, **55** : 350-354.

Bora, L. C. and Addy, S. K., 1982, Quantitative aspects of assessing virulence of *Pseudomonas syringae* in tomato plants. *Indian Phytopathol.*, **35** : 285-290.

Bose, P. C. and Singh, C., 1980, Chemical control of bacterial canker of mango. *Pesticides,* **14** : 30-31.

Bradbury, J. F., 1970, Isolation and preliminary study of bacteria from plants. *Rev. of Pl. Pathol.,* **49** : 213-218.

Chakravarti, B. P. and Rangarajan, M., 1966, Streptocycline an effective antibiotic against bacterial plant pathogens. *Hindustan Antibiot. Bull.,* **8** : 209-211.

Chakravarti, B. P., Shekhawat, P. S. and Choudhary, S. C. K., 1976, Control of bacterial blight of cowpea. *Univ. Udaipur Res. J.,* **14** : 89.

Chand, R., 1996, Grapevine bacterial canker and its management. In : *Advances in Diseases of Fruit Crops in India*, pp. 161-172.

Chand, R. and Kishun, R., 1990, Outbreak of grapevine bacterial canker disease in India. *Vitis*, **29**(3) : 183-188.

Chand, R. and Kishun, R., 1990a, Effect of temperature on the growth of grapevine bacterial canker pathogen. *Drakshavritta Souvenir,* **6** : 73-75.

Chand, R. and Kishun, R., 1990b, Outbreak of grapevine bacterial canker disease in India. *Vitis,* **29** : 183-188.

Chand, R. and Kishun, R., 1991, Inoculation techniques for *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye. *Int. J. Trop. Plant Dis.,* **9** : 115-122.

Chand, R., Kishun, R. and Singh, S. J., 1991, Characterization of bacteriophage parasitizing on *Xanthomonas campestris* pv. *viticola. Indian J. Exp. Biol.,* **29** : 180-181.

Chand, R. and Patil, B. P., 1993, Effect of GVBCD on cane health and fruitfulness in April pruning of grapevines. *Drakshavritta Souvenir,* **9** : 111-115.

Chand, R. and Ramakishun, R., 1988, April pruning and bacterial blight in grape vine. *Drakshavritta,* Souvenir, pp. 49-57.

Chand, R., Kishun and Singh, S. J., 1991b, Characterization of bacteriophage parasitizing on *Xanthomonas campestris* pv. *viticola. Indian J. Exptl. Biol.*, **29** : 180-181.

Chauhan, H. L., Vaishnav, M. U., 1980, Control of bacterial blight of rice caused *Xanthomonas campestris* pv. *oryzae. Indian J. Mycol. and Pl. Pathol.*, **10**(1) : 77-79.

Chen, R. C., Hsu, C. S. and Chiu, K. Y., 1972, Effect of several pesticides on the growth of certain plant pathogenic bacteria. *NTU Phytopathol. Entomol.,* **2** : 165-171.

*Chen, W. L., Li, D. B. and Ge, Q. *Xanthomonas*, 1990, A study on *Enterobacter cloacae* B8, *Bacillus subtilis* B826 and their antagonistic substance to *Xanthomonas campestris* pv. *oryzae. Acta Agric. Univ. Zhejiangensis,* **168** : 61-67.

Desai, S. G., Patel, M. K. and Desai, M. V., 1967, *in-vitro* activity of streptocycline against bacterial plant pathogens. *Indian Phytopathol.*, **20** : 296-300.

Dye, D. W., 1978, A proposed nomenclature and classification for plant pathogenic bacteria. *N. Z. J. Agric.,* **21** : 153-177.

Ercolani, G. L., 1968, Efficiency and measurement of transmission of *Xanthomonmas vesicatoria* and *Corynebacterium michiganense* through tomato seeds. *Industria Conserve*, **43** : 15-22.

*Gallardo, P. B., Panno, L. C. and Guichaquelen, V., 1989, Inhibition *in-vitro* of *Pseudomonas solanacearum* E. F. Smith by using the antagonist BC-8 strain of *Pseudomonas fluorescens. Revista de Microbiologia,* **20** : 27-33.

Ganacharya, N. M., 1980, Evaluation of fungicidal seed treatment for soybean. *Indian Phytopathol.*, **33** : 158.

Gaur, R. B., Ahmed, S. R. and Kataria, P. K., 1984, Controlling *Xanthomonas campestris* pv. *phseoli* Dowson of moong seed through chemotherapy and heat therapy. *Hindustan Antibiotics Bull.,* **26**(1/2) : 23-26.

Gitaitis, R. D., Bell, D. K. and Smittle, D. A., 1986, Epidemiology and control of bacterial blight and canker of cowpea. *Pl. Disease*, **70** : 187-190.

Graham, J. H., 1982, Preservation of three bacterial pathogens of soybean in culture. *Plant Dis. Rep.*, **36** : 22-23.

Gupta, D. K., 1977, Evaluation of antibiotics and fungicides against bacterial blight of guar (*Cyamopsis tetragonoloba*). *Veg. Sci.,* **4** : 25-27.

Gupta, D. K. and Chakravarti, B. P., 1983, Inoculum concentration on development of black spot and multiplication of *Xanthomonas Campestris* pv. *campestris* in cabbage. *Indian Phytopathol*., **36** : 11-13.

Halfeld-Vieira, B. A. and Nechet, K. L., 2006, Bacterial canker of grapevine in Roramia, Brazil. *Fitopatologia-Brasileria,* **31**(6) : 604.

Hayward, A. C., 1983, Preliminary diagnosis of plant disease caused by bacteria. *Plant Bacterial Disease : A Diagnostic Guide*, pp. 1-22.

Hazel, W. J. and Civerolo, E. L., 1980, Procedures for growth and inoculation of *Xanthomonas fragariae* causal organism of angular leaf spot of strawberry. *Pl. Dis.,* **64** : 178-181.

Jeyachandran, K. S. and Shanmugam, N., 1979, Studies on the control of bacterial blight of cotton. *Madras Agric. J.*, **66** : 24-27.

Karuna, K., 1993, Chemical and biological control of bacterial wilt of tomato (*Lycopersicon esculentum* Mill) caused by *Pseudomonas solanacearum* E. F. Smith. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Bangalore, Karnataka, India.

Kishun, R. and Sohi, H. S., 1982, Chemical control of bacterial spot of tomato. *Indian Phytopathol*., **35**(2) : 323-324.

Kishun, R., Singh, D. V. and Banerjee, A. K., 1979, Evaluation of chemicals for the control of bacterial leaf spot of Zinnia. *Pesticides,* **13**(2) : 51-53.

Koizumi, M., 1976b, Behaviour of *Xanthomonas citri* (Hasse) Dowson in the infection process. I. Multiplication of the bacteria and histological changes following needle prick inoculation. *Ann. Phytopathol. Soc.,* Japan, **42** : 407-416.

Kore, S. S. and Dhutraj, D. N., 1991, Detection, isolation, pathogenicity and control of seed borne bacteria of soybean. *Indian Phytopathol*., **45** : 178.

Krishna, A. and Nema, A. G., 1986, Evaluation of chemicals for the control of citrus canker. *Indian Phytopathol*., **36**(2) : 348-350.

Lapwood, D. H. and Read, P. J., 1986, The susceptibility of stems of different potato cultivars to black leg caused by *Erwinia carotovora* subsp*. atroseptica. Ann. Appl. Biol.,* **109** : 555-560.

Lenka, S., Ram, R. and Srivastava, J. S., 1997, Pathogenicity of *Xanthomonas campestris* pv. *campestris* on cauliflower (*Brassica oleracea* L. var. *Botrytis*) causing black rot. *Envion. and Ecol.,* **15**(3) : 720-721.

Mahto, B. N., Singh, R. N. and Singh, G. P., 1988, Response of rice bacterial blight pathogen *in-vitro* to antibiotics and fungi toxicants*. Int. Rice Res. News lett*., **13** : 23.

Malavolta, V. A. Jr., Almeida, I. M. G., Sugimosi, M. H., Ribeiro, I. J. A., 1999, Occurrence of *Xanthomonas campestris* pv. *viticola* in grape in Brazil. *Summa-Phytopathol*., **25**(3) : 262-264.

Mandge, A. S., Datar, V. V. and Sontakke, M. B., 1992, Chemical management of bacterial leaf spots and thirps of chilli. *J. Maharashtra Agric. Univ.,* **17** : 280-281.

Manicom, B. Q., 1986, Factors affecting bacterial black spot of mangoes caused by *Xanthomonas campestris* pv. *mangiferaeindicae. Ann. Appl. Biol.,* **109** : 129-135.

Manjula, C. P., Khan, A. N. A., Jolikop, S. H. and Ravikumar, M. R., 2007, Management of bacterial blight of pomegranate. *Environment and Ecology*, **25**(2) : 385-388.

*Mariano, R. L. R. and Gama, M. A., 2005, Preservation methods and growth of *Xanthomonas campestris* pv. *viticola* in culture medium varying temperature, pH and NaCl concentrations. *Fitopatologia-Brasileira*, **30**(6) : 650-654.

Mary, C. A. and Mathew, J., 1983, Chemical control of bacterial leaf blight of rice using antibiotics. *Agric. Res. J.*, Kerala, **21** : 17-20.

Mathur, R. L., Daftari, L. N. and Jhamaria, S. L., 1973, Controlling black arm of cotton by fungicides and antibiotics. *Indian J. Mycol. Pl. Pathol.*, **3** : 107-108.

Namasivayam, K., 1969, Studies on the black rot of crucifers caused by *Xanthomonas campestris*. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Bangalore, Karnataka, India.

Nayudu, M. V., 1972, *Pseudomonas viticola* an incitant of new bacterial diseases of grape vine. *Phytopathol. Z.,* **73** : 183-186.

Nema, A. G., Nayak, M. L. and Kulkarni, S. N., 1975, Estimation of losses by *Xanthomonas beticola* causing bacterial leaf spot of pan in Jabalpur. *JNKVV Res. J.,* **9** : 183-184.

Padgnur, G. M. and Basavaraj, M. K., 1983, Spray schedule for the control of important foliar disease and bollworms of cotton in the transitional belt of Karnataka. *Indian J. Agric. Sci.,* **53** : 725-729.

Patil, B. K., 1988, Bacterial blight of grape vine. *Drakshavritta*, **12** : 109-110.

Plessis, H. J., 1983, Chemical control of bacterial spot on plums : preliminary evaluation of bactericides. *Deciduous Fruit Growers,* **33**(11) : 413-418.

Rajpurohit, T. S. and Lodha, P. C., 1982, Note on the chemical control of bacterial blight of cotton. *Indian J. Mycol. and Plant Pathol.,* **11** : 277-279.

Raju, K. S., Rao, G. S. and Subbayu, J., 1980, Chemical control of bacterial leaf spot of chilli. *Madras Agric. J.*, **67**(2) : 825-827.

Rameshchand and Ramkishun, 1991, Inoculation techniques for *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye. *Int. J. Tropical Pl. Dis.*, **9**(1) : 115-122.

Rameshchand, Patil, B. P. and Ramkishun, 1991, Management of bacterial canker disease (*Xanthomonas campestris* pv. *viticola*) of grapevine (*Vitis vinifera*) by pruning. *Indian J. Agric. Sci.*, **61**(3) : 220-222.

Rameshchand, Patil, B. P. and Ramkishun, 1992, Efficacy of different chemicals against grapevine bacterial canker disease (*Xanthomonas campestris* pv. *viticola*). *Indian J. Pl. Protection,* **20**(1) : 108-110.

Rameshchand, Ramkishun, and Patil, B. P., 1991, Studies on grapevine bacterial canker disease-transmission and efficacy of bactericides. *Indian J. Pl. Pathol.,* **19**(1) : 97-100.

Ramkishun and Sohi, H. S., 1984, Control of bacterial canker of mango by chemicals. *Pesticides*, **18** : 32-33.

Rao, S. A. N., 1990, Protection of tomato from bacterial rot by Bacterinol-100. *Curr. Res.,* **19** : 100-101.

Ravikumar, M. R., 1996, Studies on bacterial leaf spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* in Northern Karnataka. *M. Sc. (Agri.) Thesis,* Univ. Agric. Sci., Bangalore, Karnataka, India.

Ravikumar, M. R. and Khan, A. N. A., 1995, Effect of seed treatment with chemicals and antibiotics on the viability of *Xanthomonas campestris* pv. *vesicatoria* on tomato seeds. Paper presented in *Nation. Sem. in Seed Pathol.,* held at Manasagangotri from March 23-25, 1995.

Ravikumar, M. R. and Khan, A. N. A., 2001, Field evaluation of antibiotics and chemicals against bacterial spot of tomato in Karnataka. *Pestology,* **6** : 11.

Ravikumar, M. R., Shamarao Jahagirdar and Khan, N. A., 2002, Management of bacterial leaf spot of grape through chemicals and antibiotics in northern Karnataka. Paper presented in *Nation. Symp. on Pl. Disease Scenario* in Southern India, held at GKVK, UAS, Bangalore from 19-21, December 2002, p. 65.

Ravikumar, M. R., Yenjerappa, S. T., Shamarao Jahagirdar and Srikant Kulkarni, 2006, Management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in Karnataka. *Dhalimba*, **26** : 126.

Sakthival, N., Sivaramani, E., Unnamalai, N. and Gnanamanickam, S. S., 1986, Plant growth promoting rhizobacteria in enhancing plant growth and suppressing plant pathogens. *Curr. Sci.,* **55** : 22-24.

Schaad, N. W. and Stall, R. E., 1988, *Xanthomonas*, 81-84. In : *Laboratory for Identification of Plant Pathogenic Bacteria*, 2nd Ed., American Phytopathological Society, p. 138.

Schaad, N. W. and White, R. E., 1974, *Xanthomonas*, 81-94. In : *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 2nd Ed. American Phytopathological Society, p. 138.

Shanmugavelu, K. G., 2003, Grape cultivation and processing. *Agrobios* (India), pp. 289.

Sharma, R. R., Thind and Singh, N., 1981, *In-vitro* and *in-vivo* evaluation of chemicals against *Xanthomonas vesicatoria*, the causal agent of bacterial leaf spot of chilli. *Indian J. Mycol. Pl. Pathol.*, **11** : 178-181.

Sharma, R. R., Thind, B. S. and Nirmaljit Singh, 1982, *In-vivo* and *in-vitro* evaluation of chemicals against *Xanthomonas campestris* pv. *vesicatoria* the causal agent of bacterial leaf spot of chillies. *Indian J. Mycol. Pl. Path.*, **11**(2) : 178-182.

Shekhawat, P. S., Jain, M. L. and Chakravarti, B. P., 1982, Detection and seed transmission of *Xanthomonas campestris* pv. *campestris* causing black rot of cabbage and cauliflower and its control by seed treatment. *Indian Phytopathol.*, **35**(3) : 442-447.

Shukla, B. N., Chand, J. N. and Kulkarni, S. N., 1976, Effect of leaf age on the bacterial blight of sesamum. *Indian Phytopathol.*, **28** : 304-305.

Singh, R. P. and Verma, J. P., 1973, Control of bacterial blight of cotton. *Pesticide*, **7** : 16-17.

Sinha, G. S., Parashar, B. D. and Hooda, I., 1988, Role of phenolic compounds and mineral elements in resistance of some guar cultivar to bacterial blight. *Plant Disease Res.*, **3** : 48-49.

Sivamani, E., Anuratha, C. S. and Gnanamanickam, S. S., 1989, Toxicity of *Pseudomonas fluorescens* towards bacterial plant pathogens of banana (*Pseudomonas solanacearum*) and rice (*Xanthomonas campestris* pv. *oryzae*). *Curr. Sci.*, **56** : 547-548.

Srivastava, S. S. L. and Bais, B. S., 1985, Chemical control of bacterial leaf pustule of soybean. *Indian Phytopathol.*, **38** : 351-353.

Sukhatme, P. V. and Amble, A. N., 1985, *Statistical Methods for Agricultural Workers*, Publication and Information Division, ICAR, New Delhi, p. 553.

Thapliyal, P. N. and Dubey, K. S., 1986, Chemical control of bacterial pustule of soybean. *Indian Phytopathol.*, **39** : 461-462.

Thirumalachar, M. J., Patel, M. K., Kulkarni, N. B. and Dhande, G. W., 1956, Effects of *in-vitro* evaluation of some antibiotics on thirty two *Xanthomonas* species occurring in India. *Phytopathol.,* **46** : 486-488.

Thombre, P. A., Kurundkar, B. P. and Kawale, B. R., 1989, Chemical control of bacterial pustule of soybean. *Indian Phytopathol.* (Abstr.), **42** : 234.

Thirumurti, V. S. and Agarwal, K. C., 1992, Efficacy of Streptocycline in controlling bacterial pustule (*Xanthomonas campestris* pv. *sojense*) of soybean. *Indian J. Pl. Pathol.,* **22** : 190-191.

Vajawat, R. M., Chakravarti, B. P. and Jain, K. L., 1985, Inoculum concentration on symptom development, pathological changes after infection and multiplication of *Pseudomonas syringae* pv. *sesami* in field grown sesame of different ages. *Indian J. Mycol. Pl. Pathol.,* **15** : 141-144.

Vasantha Kumar, G. K., 2007, Status of viticulture in Karnataka – Past present and future. Paper presented at the *Int. Symp. on Grape Production and Processing*, at Baramati, Maharashtra (India), held on February 6-11, 2006.

Venugopal, M., 1983, Studies on variation in *Xanthomonas campestris* pv. *mangiferaeindicae* (Patel *et al*.) Robbs et al. The causal organism of bacterial canker of mango. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Bangalore, Karnataka, India.

Verma, J. P., Singh, R. P. and Nayak, M. L., 1976, Laboratory evaluation of chemicals against *Xanthomonas malvacearum* the incitant of bacterial blight of cotton. *Indian Phytopathol.*, **28** : 171-174.

Viana, I. O., 2006, Medium for isolation of *Xanthomonas campestris* pv. *viticola. Ciencia-Rural,* **36**(4) : 1317-1320.

Wet, T. H. De., 1982, Bacterial spot and blossom malformation. *Mango Grower's Assoc.*, **2** : 18-22.

Wheeler, B. E. J., 1969, *An Introduction to Plant Diseases*, John Wiley and Sons Ltd., London, p. 301.

**Originals not seen*

STUDIES ON BACTERIAL LEAF SPOT OF GRAPE CAUSED BY *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye IN NORTHERN KARNATAKA

SHIVANANDA JAMBENAL 2008 Dr. M. R. RAVIKUMAR
Major Advisor

ABSTRACT

Among the several diseases, bacterial leaf spot is one of the most destructive diseases of grape, which causes considerable loss in yield and quality of produce. The present investigations were undertaken, survey for bacterial leaf spot of grape in Northern parts of Karnataka, isolation, identification and pathogenicity studies. To evaluate different chemicals, bioagents and their combinations both under *in-vitro* and *in-vivo* conditions.

Surveyed during 2007-08 both at April and October pruning stages revealed that the disease was severe during April pruning stage as compared to October pruning stage. The places like Tikota (Bijapur district) and Chikkapadasalagi (Bagalkot district) were identified as ‘hot spots’ for this disease.

The bacterium was isolated from infected leaves showing typical symptoms of bacterial spot, yielded yellow, mucoid, shiny, slimy, convex colonies on nutrient agar medium.

Based on the physiological, biochemical and morphological characteristics, the bacterium was identified as *Xanthomonas campestris* pv. *viticola*. Further, hypersensitive reaction on *Nicotiana tabaccum* var. *samsun* and pathogenicity test on grape plants, the bacterium was identified as *Xanthomonas campestris* pv. *viticola*.

Among the various bactericides, antibiotics, bioagents alone and their combinations were tested under *in-vitro* condition, the streptocycline 500 ppm plus copper oxychloride 2000 ppm and *Bacillus subtilis* (bioagent) were produced maximum inhibition zone against *Xanthomonas campestris* pv. *viticola*.

Different chemicals, bioagents alone and their combinations were evaluated under field condition revealed that, streptocycline 500 ppm plus copper oxychloride 2000 ppm were effectively controlled the disease (incidence) which was recorded very less per cent disease index and significantly superior over all the other treatments. Among the bioagents, *Bacillus subtilis* 5000 ppm was gave good result.

The yield and yield parameters like number of branches production and number of bunches infected per plant, weight of single bunch were significantly superior in case of streptocycline 500 ppm plus copper oxychloride 2000 ppm.