MANAGEMENT OF BACTERIAL BLIGHT OF POMEGRANATE CAUSED BY *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vauterin *et al.*

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CONTENTS

Contd…

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family punicaceae. Pomegranate is a native of Iran, where it was first cultivated in about 2000 BC, but spread to the Mediterranean countries at an early date. It is extensively cultivated in Spain, Morocco and other countries around the Mediterranean, Egypt, Iran, Afghanistan, Arabia and Baluchistan. It is also grown in Burma, China, Japan, USA, USSR, Bulgaria and Southern Italy.

The fruit is very much liked for its cool and refreshing juice. The arils of the well matured fruits are consumed as such and also in processed form like juice or concentrate, syrup and jelly. Seeds with fleshy portions of sour pomegranates are dried and marketed as 'Anardana', which is being used as a condiment and for souring curries.

Pomegranate is a good source of carbohydrates and minerals such as calcium, iron and sulphur. It is rich in vitamin-C and citric acid is the most predominant organic acid in pomegranate (Malhotra *et al*., 1983). Glucose (5.46%) and fructose (6.14%) are the main sugars with no sucrose in fruits. The fruits of pomegranate are known to pharmaceutical and therapeutic values. Sweet varieties are mildly laxative, sour types are good for curing inflammation of stomach and heart ache. In India, there is a common adage 'Ek Anar Sau Bimar' meaning is one fruit cures hundred diseases. The flower buds are very useful in ayurveda for managing bronchitis. The bark of the stem, root and rind of the fruit is used for slimming, control of dysentery, diarrhoea and killing tape worms.

Seeds with fleshy portions of sour pomegranates are dried and marketed as "Anardana", which is being used as a condiment for curries. Fruits are the important raw materials for wine industry because of easy fermentation. Other value added products are juice, jelly, anarub and rind powder.

Apart from the fleshy portion of the fruit, the crop residues are also finding place in industries. The rind of the fruit is a good source of dye, which gives yellowish brown to khaki shades and is being used for dying wool and silk. The flower and buds yield light red dye, which is used for dying of cloths in India. The bark (stem) and rind (fruit) portion is used to treat diarrhoea and indigestion (Anon., 1969). The bark of the stem and root contains number of alkaloids belonging to pyridine group. The bark is also used in tanning industry (Patil and Karle, 1990).

The most popular varieties suitable for processing and table use are Ganesh, Mridula, Arakta, Bhagwa (Kesar), G-137 and Khandar. Area under pomegranate is increasing worldwide because of its hardy nature, wider adaptability, drought tolerance, higher yield levels with excellent keeping quality and remunerative prices in domestic as well as export market. It thrives well in dry tropics and sub-tropics and comes up very well in soils of low fertility status as well as on saline soil.

Pomegranate is regarded as the "Fruit of Paradise". It is one of the most adaptable subtropical minor fruit crops and its cultivation is increasing very rapidly. In India, it is regarded as a "vital cash crop", grown in an area of 1.5 lakh ha with a production of 11.0 lakh tones (Jadhav and Sharma, 2009). Among the different states growing pomegranate, Maharashtra is the largest producer occupying $2/3^{rd}$ of total area in the country followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. Karnataka state has the distribution of cultivating pomegranate under tropical condition in an area of 12,042 ha with a production of 1,29,547 tonnes (www.horticulture.kar.nic.in). In Karnataka, this crop has spread across different districts *viz*., Bijapur, Bellary, Bagalkot, Koppal, Chitradurga, Belgaum, Davangere, Tumkur, Bangalore and Gulbarga. Successful cultivation of pomegranate in recent years has met with different traumas such as pest and diseases. Among diseases bacterial blight caused by *Xanthomonas axonopodis* pv. *Punicae* (Vauterin et al., 1995) is a major threat. Since 2002, the disease has reached the alarming stage and hampering the Indian economy vis-à-vis export of quality fruits. The disease accounted up to 70 – 100 per cent during 2006 in Karnataka and Maharashtra resulting in wipe out of pomegranate during the year 2007, the total output of pomegranate production in India was down by 60 per cent (Raghavan, 2007).

For the first time in India, leaf spot of pomegranate was reported by Hingorani and Mehta (1952). Later on during 1959, Hingorani and Singh took a thorough investigation on the disease and causal organism and designated the pathogen as *Xanthomonas punicae* sp. Nov. Chand and Kishun (1991) reported the epidemic form of disease in Bangalore caused 60 – 80 per cent loss during 1991 and they also isolated the causal organism and identified it as *Xanthomonas campestris* pv. *punicae* based on its pathological, cultural, biochemical and physiological characters. Later, on the basis of DNA homology, the pathogen has been renamed as *Xanthomonas axonopodis* pv. *punicae* (Vauterin *et al.,* 1995).

The production of pomegranate has declined from a high of 1.8 lakh metric tonnes per annum four years ago to less than 10,000 metric tonnes in 2007 – 08, there by causing a revenue loss of about Rs. 200 crore (at an average price of Rs. 50,000 per tonne) in Karnataka (Giridhar, 2008). Survey report in Karnataka revealed that, 20 – 90 per cent of disease severity in Bijapur and Bagalkot districts (Ravikumar *et al*., 2006), and recent reports revealed the highest severity of tree was 74.80 per cent in Bagalkot district and minimum severity of 6.73 per cent in Bellary districts (Anon., 2008). During 2008 – 09 the disease has reached its alarming stage bringing substantial damage to the crop and heavy loss to the farmers. However, the magnitude of the disease severity has raised several questions to researchers, farm managers, administrators, private firms and above all the farmers to safeguard the cultivation of this dollar earning crop.

Disease is characterised by the appearance of small, irregular water soaked, dark coloured spots on leaves resulting in premature defoliation. Pathogen also infects stem and branches causing girdling and cracking symptoms. Spots on fruit are dark brown, irregular slightly raised with oily appearance, which split opens with ‘L’/‘Y’ shaped cracks at final stages. Under severe condition it destroys the entire orchard and causes heavy economic losses.

There is little information available on management of bacterial blight of pomegranate, but there are large number of chemicals available in the market as bactericides and their bioefficacy and suitability needs to be verified by *in vitro* and field studies, so as to incorporate the effective ones in the management package.

In recent years, there has been a major thrust on residue free organic pomegranate production. Taking the task into consideration, efficient botanicals and bioagents need to be explored to fit into the management schedule.

Keeping all these aspects in view, the investigation was undertaken with the following objectives.

1. Survey for the bacterial blight of pomegranate in major growing areas of north Karnataka
2. Isolation , identification and pathogenic studies
3. *In-vitro* evaluation of botanicals, bioagents and antibiotics against *Xanthomonas axonopodis* pv *punicae*
4. Management of bacterial blight of pomegranate under field condition.

2. REVIEW OF LITERATURE

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*, once deemed as a disease of minor importance, became serious threat for pomegranate cultivation in recent years. The disease assumed severity in all the pomegranate growing areas of Maharashtra, Karnataka and Andhra Pradesh resulting in severe yield loss both in terms of quality and quantity. The information available on this disease and pathogen is very meagre. Hence, the literature pertaining to the bacterial blight of pomegranate along with information on related crops, disease and pathogen are reviewed here as under.

2.1 Report on occurrence

For the first time in India, Hingorani and Mehta (1952) reported the occurrence of leaf spot of pomegranate. Later, Hingorani and Singh took thorough investigation of the disease and pathogen and reported the presence of disease in different parts of the country.

This disease was also reported from Annamalainagar of the then Madras state (Rangaswamy, 1962) and Solan region of Himachal Pradesh (Sohi *et al.,* 1964).

First record of bacterial blight on pomegranate in Karnataka was by Chand and Kishun (1991). They reported the severe incidence of the disease causing 60 to 80 per cent losses at IIHR experimental plots in Bangalore.

Outbreak of the disease was noticed in major pomegranate growing areas of Bellary and Bijapur districts causing severe loss both in terms of yield and quality (Anon, 2002). Pomegranate, the commercial crop of the farmer turned as a bane after the outbreak of bacterial blight.

2.2 Survey of bacterial blight of pomegranate

Sudarshan Rao (1975) stated that, survey and surveillance form the basis for any successful plant protection strategy. Plant protection to be successful, depends upon early detection of the disease followed by timely adoption and application of preventive measures.

Kanwar (1976) took the survey in orchards of Haryana and observed the new disease on pomegranate caused by *Xanthomonas punicae*.

Survey of grapevine nurseries for bacterial canker incidence in various regions of Maharashtra state over the years (1987-1990) indicated that, all the nurseries located in Ahmednagar, Nasik, Pune and Solapur districts produced infected planting material. In 1990, nine out of ten locations showed infection ranging from 85 to 100 per cent (Chand *et al.*, 1991).

Manjula and Khan (2002) made the survey on bacterial blight of pomegranate and reported that, the disease appeared in a devastating form in Bellary, Bijapur and Bangalore districts of Karnataka during late summer and *kharif* season of 2001. The pathogen infected all the cultivated varieties of pomegranate irrespective of age of the plants and resulted in severe yield loss.

Ravikumar *et al.* (2004) took the survey for the bacterial diseases infecting fruits and vegetables in Bagalkot and Bijapur districts. They recorded 20–90 per cent of bacterial blight on pomegranate, 10–81 per cent leaf spot on tomato and 1–20 per cent of tip over disease on banana. The citrus canker was found as the major production constraint in citrus with an incidence ranged between 20–60 per cent.

To study the bacterial blight severity of pomegranate and its impact on crop losses, survey was conducted by Yenjerappa *et al.* (2004) in Bellary district and indicated that, disease was severely noticed to the tune of 71.4 per cent in major pomegranate growing villages of Bellary taluk and 53.8 per cent incidence in Bellary adjacent villages of Andhra Pradesh. Disease symptoms were very conspicuous on leaf, stem and fruits. All the affected leaves were dropped, breaking of stem and fruit cracking was observed in severely affected gardens.

Roving survey was conducted by Benagi *et al.* (2009) during mrigbahar and hastabahar (August to January) of 2008-09 in major pomegranate growing areas of north

Karnataka. Plantations of age three years and above were selected for the survey and reported per cent severity ranged between 0.67 - 94.80. The disease was very severe in Bagalkot district with an average severity value of 74.08 per cent. The least average severity (6.73%) was observed in Bellary district.

2.3 Symptomatology

Hingorani and Mehta (1952) described symptoms as irregular leaf spots, varying from 2.0 mm to 5.0 mm in diameter and later adjacent spots coalesce and cover larger areas. The spots were initially light brown in colour, surrounded by water soaked margin, later turned into dark brown as the disease progressed. The formation of several spots on leaf induced shedding and spots were also noticed on twigs.

Rangaswamy (1962) observed the symptoms of bacterial blight on the leaves as necrotic spots surrounded by chlorotic halos with translucent water soaked appearance. Under severe infections, leaves became distorted and shed off. Fruits also get infected with water soaked spots in the earlier stages, later became dark brown, slightly raised from the surface with oily appearance.

Kanwar (1976) observed small, brown, water soaked spots on leaves, flowers and fruits of pomegranate in different orchards of Haryana. Initially, spots on leaves were small, circular with yellowish border and brown centre. Later on, number of adjacent spots coalesce to form irregular lesions. In many cases, spots were restricted on leaves by veins, resulting in linear stripes. Tissue necrosis and defoliation occurred in advanced cases of infection. On petals of flowers, small, brown, water soaked spots were seen, which later became dark and bigger in size. Symptoms were also noticed on immature fruits as small, pin head like, circular with dull green colour, which later turned to deep brown or black with yellowish zone surrounding the spots. As the disease advanced, spots on fruit coalesce to form irregular lesions resulting in rough skin. Fruits remained underdeveloped and deformed under severe infection.

Kishun (1993) reported similar kind of symptoms on leaves, but on stem, the disease appeared as brown to black coloured spots around the nodes leading to girdling and cracking of nodes. Brown to black spots also appeared on fruit pericarp, which later turned to 'L' or 'Y' shaped cracks.

Manjula and Khan (2002) described the symptoms as minute water soaked lesions both on leaves and fruits, which later turned brown to black coloured spots surrounded by diffused water soaked margin. The necrotic lesion on the fruit increased as the fruit develops with age leading to L, Y or Star shaped cracking within the spots. Severely infected fruits split opens, exposing the arils. Bacterial lesions also noticed on twigs/branches resulting in death of the branches. Affected plants looked weak and died later on (Madhu, 2009).

2.4 Isolation and pathogenicity

Hingorani and Mehta (1952) isolated bacterial leaf spot pathogen from the infected pomegranate leaf using dilution plate technique and proved pathogenicity by spray method of inoculation in absence of wounds. Infection readily occurred on the tender leaves of artificial inoculated young potted plants, after 7-10 days of inoculation. Isolation from the infected plants yielded an organism similar to one used in the inoculation experiments.

Isolation of the bacterial blight pathogen of pomegranate was made within 48 hours after collection of samples on nutrient dextrose agar medium. All the diseased samples yielded yellow pigmented colonies after 3-5 days of inoculation at 25-30ºC temperature. Pure cultures were established by repeated single colony transfer method. For pathogenicity test, inoculation was made by spraying bacterial suspension of 48 hour old culture onto the injured and uninjured leaves, flowers and fruits of healthy plants. Infection occurred more rapidly on injured leaves, flowers and fruits than uninjured plants. Organism reisolated from artificially inoculated plant was found identical with original one (Kanwar, 1976).

Chand and Kishun (1991) standardised the inoculation method for *Xanthomonas axonopodis* pv. *punicae* by inoculating the bacterial suspension (2×10^{8}cfu/ml) on 40 days old leaves by pinprick, rubber block pressure, leaf cut and automization methods. They observed that, leaf cut was found superior, where they recorded 100 per cent infection covering 70 to

90 per leaf area within 21 days. The automization of bacterial suspension was found to induce lowest infection of 6 - 7.5 per cent with maximum incubation period of 17 to 40 days.

Manjula (2002) obtained the pure culture of the seven isolates of the *X. axonopodis* pv. *punicae* from infected leaf, twig and fruit samples of pomegranate collected from different areas of survey. She has isolated the pathogen on nutrient agar medium by dilution plating technique and pathogenicity of all the seven isolates were proved by spray method of inoculation using 45 plants of variety Jyothi.

2.5 Identification of the pathogen

Hingorani and Singh (1959) described the pathogen as *Xanthomonas punicae* sp. Nov., which has a short rod with rounded ends; single or in pairs; sometimes in chains; no involution forms; 1 - 2.5 × 0.5 µm in size; motile with a single polar flagellum; Gram-negative; non-endospore forming; capsulated and non-acid fast.

According to Kanwar (1976) the bacterial blight pathogen of pomegranate was seen in single pairs and also in chains. It was rod shaped with rounded ends and measured 0.75 to 3.0 µm in length and 0.45 µm in width, Gram negative, with single polar flagellum, neither capsules nor endospores were observed.

2.6 Evaluation of bactericides / antibiotics

2.6.1 *In vitro* studies

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*.

In vitro evaluation of eight chemicals against *Xanthomonas vesicatoria* by paper disc and turbidometric methods was carried out by Sharma *et al.* (1981). They described that, combination of streptocycline and copper sulphate was most effective in inhibiting the growth of pathogenic organism in turbidometric method, but stable bleaching powder showed maximum inhibition, when tested by Thompason's method.

Venugopal (1983) studied *in vitro* sensitivity of different isolates to antibiotics by paper disc method against *Xanthomonas campestris* pv. *mangiferaeindicae*. Isolates obtained from varieties such as Raspuri, Bappukai and Lalbaugh exhibited sensitivity to streptomycin and paushamycin @ 100 and 250 ppm, respectively.

Krishnan and Madhumeeta (1988) observed that plantomycin, streptocycline and paushamycin each at 500 ppm concentration were equally effective in inhibiting the growth of *Xanthomonas campestris* pv. *dieffenbachiae* causing bacterial blight of anthurium.

The efficacy of bactrinol 100 (2 bromo 2 nitro propane 1, 3 diol), a novel antibacterial agent was tested against *Xanthomonas campestris* pv. *oryzae* (Natarajan and Lalithakumari, 1991). The results indicated that, the chemical had little effect on the rate of oxygen uptake, but rate of electrolytic leakage on bacteria was increased considerably. The total DNA or RNA contents were not affected appreciably. However, protein and lipid contents decreased significantly. Both the quality and quantity of free amino acid pool were altered by bacterinol-100 treatment.

Results obtained by Manjula *et al.* (2002) on the *in vitro* efficacy of bactericides against *Xanthomonas axonopodis* pv. *punicae* indicated that paushamycin (500 ppm) and K cycline (500 ppm) produced the highest inhibition zone followed by bacterinol and bacteriomycin. Copper oxychloride @ 2000 ppm was found moderately effective and kasugamycin @ 500 ppm concentration was ineffective.

Yenjerappa (2009) reported that bactinash-200 (15.07 mm), bronip (14.67 mm) and plantomycin (13.77 mm) exhibited significantly superior efficacy in inhibiting the growth of *Xanthomonas axonopodis* pv. *punicae*. The other bactericides *viz.,* K cycline, copper oxychloride, Bordeaux mixture, bactinashak, bleaching powder and streptocycline were found moderately effective with an inhibition zone ranged from 8.40 to 10.23 mm. In respect of concentration, efficacy of each chemical was significant at relatively higher concentrations.

2.6.2 Field evaluation

Kishun and Sohi (1984) reported that four sprays of bavistin (1000 ppm) or bavistin (1000 ppm) + agrimycin 100 (100 ppm) at monthly intervals were significantly effective in controlling the bacterial canker of mango. Among the four fungicides/bactericides tested under field condition by Gupta (1991) revealed that all the four chemicals *viz.*, agrimycin 100 (1000 ppm), streptocycline (250 ppm), carbendazim and benomyl each at 500 ppm concentration were equally effective and significantly superior over untreated plot in checking the black rot menace on cabbage.

Suriachandraselvan *et al.* (1993) reported that, the application of paushamycin (0.05%) along with copper oxychloride (0.2%) was most effective in controlling the disease on pomegranate caused by *Xanthomonas campestris* pv. *punicae*. Atulchandra *et al.* (1994) stated that, same bacterium could be controlled by spraying of Bordeaux mixture (5:5:50) or any other copper fungicides at an interval of 15 days.

Manjula *et al.* (2002) obtained the effective control of bacterial blight in pomegranate with the sprays of either paushamycin or streptocycline or K-cycline each sprayed individually at 500 ppm concentration. Bacterinol 100 was also found promising against the disease.

Least bacterial leaf spot incidence on grape vine was recorded in the plots sprayed with streptocycline (0.05%) or streptomycin sulphate (0.05%) by Ravikumar *et al.* (2002). Pruning of infected parts along with one spray of copper oxychloride followed by four sprays of streptocycline (100 ppm) + Copper oxychloride (0.3%) found very much promising in reducing the incidence of bacterial canker of acid lime (Gopal *et al.*, 2004).

Yenjerappa *et al.* (2004) noticed the superior efficacy of streptocycline (0.05%) in combination with copper oxychloride (2000 ppm) in checking the bacterial blight menace of pomegranate.

Ravikumar and Yenjerappa (2005) found that, five sprays of bactrinashak (500 ppm) in combination with copper oxychloride (2000 ppm) was significantly effective in reducing the bacterial blight of pomegranate. Highest yield and maximum benefit cost ratio was recorded with the same treatment. Pathak and Godika (2006) opined that, delinted seed treatment with streptocycline (100 ppm) for two hours followed by spraying of streptocycline with copper oxychloride, two times, once at the time of disease onset and another after 20 days is the best practice for the management of bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum*.

Ravikumar *et al.* (2009) reported that sprays with strepto cycline (500ppm) + copper oxychloride (2000ppm) were found very effective in reducing mean disease incidence (25.5%) follwed by Bromopal (500ppm) + copper oxychloride (2000ppm) (33%), when compared with control (78.5%) after the 8th spray. The maximum mean yield of 9.3 tons/ha was recorded in strepto cycline (500ppm) + copper oxychloride (2000ppm) follwed by 8.50 tons/ha Bromopal (500ppm) + copper oxychloride (2000ppm) as against 2.95 tons/ha in untreated check.

Yenjerappa *et al.* (2009) reported that bronip, bactnash-200 in combination with COC were significantly and highly effective in reducing both incidence and severity of the disease followed by bactrinashak + COC and streptocycline + COC.

2.7 Biological control

2.7.1 Effect of biocontrol agents under *in vitro* conditions

Unnamalai and Gnanamanickam (1984) reported the inhibiting effect of *Pseudomonas fluorescens* on the growth of *Xanthomonas citri*.

Sivamani *et al.* (1987) examined the toxicity of *Pseudomonas fluorescens* towards bacterial plant pathogens of banana (*Pseudomonas solanacearum*) and rice (*Xanthomonas campestris* pv. *oryzae*). They opined that native strains of *Pseudomonas fluorescens* could be the effective biocontrol agents against *Pseudomonas solanacearum* and *Xanthomonas oryzae*.

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

Laha *et al.* (1992) stated that, fluorescent pigments produced by *Pseudomonas* were sequester Fe^{3+} and were considered as siderophores, which inhibits the large number of phytopathogenic bacteria and fungi, whereas biochemical studies by Valasubramanian *et al.* (1994) showed that, efficient strains of *Pseudomonas fluorescens* produces an antibiotic phenazine-1-carboxylic acid (PCA), which hinders the growth of plant pathogenic bacteria.

Three species of bacteria *viz*., *Bacillus subtilis*, *Bacillus polymixa*, *Pseudomonas fluorescens* and four species of fungi *viz*., *Aspergillus terreus*, *Trichoderma viridae, Trichoderma harzianum, Serratia marcescens* isolated from the phylloplane of citrus variety Assam lemon (citrus lemon) inhibited the growth of *Xanthomonas campestris* pv. *citri*, the incitant of citrus canker, when tested by agar plug method *in vitro* (Kalita *et al*., 1996). Among these bioagents, *Bacillus subtilis* was found most effective antagonist producing largest inhibition zone followed by *Pseudomonas fluorescens* and *Aspergillus terreus*.

Manjula (2002) tested the antagonistic effect of *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Lactobacillus* spp. on the growth of *Xanthomonas axonopodis* pv. *punicae* by both inhibition assay (paper disc) and dual culture plate method and observed that none of these antagonists were inhibitory to the growth of the pathogen.

Dutta *et al.* (2005) observed that, among the twenty one rhizobacterial and fifty two phylloplane bacterial isolates of mungbean, the isolates MRb-1 (rhizobacteria), Plb-1, Plb-2 and Plb-3 (phylloplane bacteria) exhibited the maximum inhibition of *Xanthomonas axonopodis* pv. *vignaeradiatae* tested by dual culture technique using sucrose peptone agar. MRb-1 was the potential antagonist followed by Plb-3, Plb-2 and Plb-3. Based on the morphological and biochemical characteristics, MRb-1 isolate was identified as *Pseudomonas fluorescens* and Plb isolates were identified as *Bacillus* spp.

Yenjerappa (2009) studied that among the different antagonists *Bacillus subtilis* and *Pseudomonas fluorescens* were found significantly superior over other antagonists in inhibiting the growth of the pathogen. The fungal biocontrol agents *viz., Trichoderma viridae* and *Trichoderma harzianum* were found totally ineffective.

2.7.2 Field efficacy of biocontrol agents

Kalita *et al.* (1996) examined the reduction of canker disease on citrus, when phylloplane species of antagonists *viz*., *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus polymixa* were applied onto the citrus crop foliage of variety Assam lemon. They recorded least canker incidence with the treatment by *Bacillus subtilis*.

The efficacy of *Pseudomonas fluorescens* in reducing the bacterial blight intensity of rice was reported by Kaur and Thind (2002). Among the three isolates of *Pseudomonas fluorescens* tested in glasshouse and field, the isolate *Pseudomonas fluorescens* LR (Ludhiana rhizosphere) proved as most effective antagonist in significantly reducing the disease intensity. Among the different methods of antagonist application tried, seed bacterization followed by two foliar sprays recorded the lowest bacterial blight intensity both in glasshouse and field conditions.

Lodha (2001) studied the effect of application of two phylloplane antagonists, a white sterile fungus and a bacterium *Bacillus subtilis* against bacterial blight of cluster bean and noticed the superiority of white sterile fungus in reducing the maximum disease intensity followed by *Bacillus subtilis*. Combined treatment of both of these antagonists in a sequence could not reduce the blight incidence significantly rather increased it.

Characterization and evaluation of native strains of rice associated *Bacillus* strains on rice that substantially suppressed the bacterial blight and increasing the tiller number and grain yield were described by Vasudevan and Gnanamanickam (2002). The strains included *Bacillus cereus, B. circulans, B. lentus* and two strains of *Bacillus* spp. Two net house and a field test in Kerala with the application of these strains suppressed the incidence of bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* by more than 50 per cent in popular rice cultivars IR-24 and Jyothi.

2.8 Effect of botanicals/plant extracts against the pathogen and disease

2.8.1 *In vitro* studies

Grainge *et al.* (1985) reported *in vitro* efficacy of aqueous extract of two plant species *viz.*, *Allium sativum* (Garlic) and *Artabotrys uncinatus* (leaves) against *Xanthomonas campestris* pv. *oryzae*.

Prasad and Alankara Rao (1987) evaluated the antimicrobial effects of essential oils of five species of *Ocimum*. All the samples showed antibacterial activity against gram positive and gram negative bacteria.

Srinivasachary (1995) found that, *Ocimum* plant extract was more effective in inhibiting the growth of *Xanthomonas campestris* pv. *mori* isolated from mulberry under *in vitro* followed by *Bursara, Citronella* and *Cinnamom*, whereas garlic extract was highly inhibitory to the growth of *Xanthomonas campestris* pv. *dieffenbachiae* followed by *Ocimum* and *Citronella* (Sushma Joseph, 1997).

Manjula (2002) observed the significant difference in the inhibitory effect among the eight plant extracts screened against the growth of *Xanthomonas axonopodis* pv. *punicae.* Kolangi extract (1:1) was found more effective against the growth of Bangalore fruit isolate followed by meswak, tulsi and patchouli, whereas meswak exhibited highest inhibitory effect followed by kolangi and patchouli on the growth of Bijapur isolate. Extracts of *Adathoda, Isabgol, Annona* and Neem seed had no positive effect against the pathogen.

Results obtained by Tiwari *et al.* (2004) on the evaluation of medicinal herbs and shrubs for their efficacy against *Xanthomonas campestris* pv. *campestris* indicated that, out of 905 plant species screened, 70 species were found to possess strong antimicrobial properties by hindering the complete growth of the pathogen at 1 per cent concentration. Some of those plant species, which exhibited superior efficacy were, *Clitoria termatea, Datura alba, Canavalia gladiata, Ahytilon indicom, Cassia occidentalis, Abelmoschus molchutus, Desmodium triangulare etc.*

Yenjerappa (2009) reported that, garlic extract at 10% concentration was significantly greater in efficacy than all other treatments followed by parthenium and lantana leaf extract and onion bulb extract each at 10% concentration.

2.8.2 Field efficacy of botanicals

Kiran Kumar (2000) reported that, *Ocimum sanctum* effectively controlled bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv. *vignicola*, when undiluted extract is sprayed onto the crop and upon dilution effectiveness was decreased.

Madhiazhagan *et al.* (2002) studied the field efficacy of five botanical extract in controlling the bacterial bight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* through pot culture experiment and mentioned that, among the five plant extracts, *Adhatoda vasica* significantly minimized the disease with higher grain yield levels. *Curcuma longa* and *Allium cepa* were the next best effective treatments.

Ashokumar (2006) reported that, crude leaf extracts (1%) of *Lantana camara, Eucalyptus citroda, E. odenophorum* and *Agave Americana* were effective in reducing the incidence of bacterial blight of rice in susceptible cultivar Kasturi.

3. MATERIAL AND METHODS

In the present investigation laboratory studies on isolation and pathogenecity of the pathogen *Xanthomonas axonopodis* pv. *punicae* were carried out in the Department of Plant Pathology, College of Agriculture, UAS, Dharwad. The details of materials used and methodology followed during the course of investigation described here under.

3.1 General procedure

In all the experimental studies Borosil glasswares were used. The glasswares were kept in the cleaning solution containing 60 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.

All the glasswares used in the study were sterilized in an autoclave at 1.1 kg per cm^2 pressure for 20 minutes and kept for drying in hot air oven at 160^0C for one hour. Both solid and liquid media were sterilized at 1.1 kg per cm^2 pressure for 15 minutes

3.2 Survey for the incidence and severity of bacterial blight in pomegranate

Roving survey was conducted during 2009 and 2010 to know the incidence and severity of bacterial blight in major pomegranate growing areas comprising Bagalkot, Bijapur, Koppal, Gadag and Raichur districts of Karnataka. Survey was taken up in the two cropping seasons *viz.,* Hastbahar and Ambiabahar on major varieties, Bhagwa and Ganesh. Incidence and severity of the disease on fruit and foliage was recorded. Number of bacterial lesions on stem were also counted and recorded. The disease severity was recorded by using the following scale developed by Anonymous (2006).

Severity of bacterial blight pomegranate was recorded by using 0-5 and 0-6 scale on leaf and fruit respectively.

Grade	Per cent infection	
	Leaf	Fruit and Stem
0	0.00	0.00
1	Upto 1	Upto 1
2	>1- 10	>1-10
3	>10-20	>10-20
4	>20-40	>20-40
5	> 40-100	>40-70
6	-	>70-100

Per cent incidence and Per cent disease index (Wheeler, 1969) on leaves, stem and fruit was calculated by applying the formula given below.

$$\text{Leaf incidence (\%)} = \frac{\text{Number of Infected leaves}}{\text{Total leaves observed in a set}} \times 100$$

$$\text{Severity (\%)} = \frac{\text{Number of leaves x disease grade}}{\text{Total leaves observed in a set x Max. grade}} \times 100$$

$$\text{Fruit incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total fruits observed in a set}} \times 100$$

$$\text{Severity (\%)} = \frac{\text{No. of fruits x disease grade}}{\text{Total fruits observed in a set x Max. grade}} \times 100$$

$$\text{Twig incidence (\%)} = \frac{\text{No. of stems or twigs infected}}{\text{Total stems observed in a set}} \times 100$$

$$\text{Severity (\%)} = \frac{\text{No. of stems or twigs x its grade}}{\text{Total stems observed in a set x Max. grade}} \times 100$$

Severity on a tree is calculated by using the formula,

Severity on tree = 0.10LB + 0.70 FB + 0.20 SC.

Where, LB- severity on leaves,

FB- severity on fruits,

SC- severity on stems.

% reduction over control:

$$\text{Disease reduction (\%)} = \frac{\text{PDI in control - PDI in treatment}}{\text{PDI in control}} \times 100$$

Where PDI - Percent disease index.

3.2.1 Isolation of the pathogen

The different parts of the pomegranate plant showing characteristic symptoms of bacterial infection were collected from different locations and subjected to isolation. The infected parts were washed in running tap water and infected portion along with small portion of healthy tissue was cut into 5 mm bits. These bits were surface sterilized with 1 per cent sodium hypochlorite and washed in three series of sterile distilled water to remove traces of sodium hypochlorite. The bits were then crushed in 2 to 3 ml of sterile distilled water and allowed to diffuse for 5 to 10 min at room temperature. A loopful of the crushed leachate was streaked on Yeast-Dextrose- Calcium carbonate agar (YDCA) plates aseptically and incubated at temperature ($30 \pm 1^{0}C$) for 2 days. Colonies grown within 48 hrs were picked out and again streaked on YDC agar plates, discrete colonies were subcultured on YDC agar slants for further studies.

The cultures were renewed by sub-culturing once in a fortnight on YDC agar slants and such cultures did not show any variation in cultural characters even after several generations of subculturing.

3.2.2 Proving pathogenicity

Detached leaf inoculation technique was followed to prove the pathogenicity (Tuite, 1969). Three middle aged leaves were selected and detached from the plants. They were washed well in tap water, swabbed with 70 per cent ethanol and allowed to dry. Then injuries were made at several points by pricking with sterilised needle charged with 10^{9}cfu/ml inoculum of *Xanthomonas axonopodis* pv. *punicae* and also smeared on both sides with culture soaked sterilised cotton swab and the petioles were dipped in 2 per cent sucrose solution. The leaves were kept in plates which were lined with sterilized moist filter paper to maintain humidity and incubated at $30^{0}C$.

3.3 Evaluation of chemicals

3.3.1 *In vitro* evaluation of chemicals

Chemicals each at three concentrations were evaluated for their efficacy against the growth of *X. axonopodis* pv. *punicae* by inhibition zone assay method.

The list of the chemicals as follows

Sl. No.	Chemical name	Trade name	Concentration (ppm)
1.	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	Streptocycline	250,500,750
2.	2-Bromo-2-Nitro Propane-1,3,-Diol	Bacterinol	250,500,750
3.	Copper Oxy Chloride 50% W.P	Blitox	1500,2000,2500
4.	Copper Hydroxide	Kocide	1500,2000,2500
5.	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper Oxy Chloride	Streptocycline+ Blitox	250,500,750 + 1500,2000,2500
6.	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper Hydroxide	Streptocycline + Kocide	250,500,750 + 1500,2000,2500
7.	Quat – NX (extract of Barberry, *Tamarindus indica, Randia dumatorum* and *Terminalia chebula*	Pathonil	2500, 5000,7500

The bacterium was multiplied by inoculating the culture into the 20 ml of nutrient broth taken in 'Erleyenmayers' flask. The inoculated flasks were incubated at 30^0C for 72 hours. The bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized petriplates and plates were allowed to solidify.

The bactericides solution were prepared at different concentrations as mentioned in the list. The filter paper discs (Whatman No. 42) measuring 5 mm in diameter were soaked in the respective chemical solution for 5 minutes and transferred onto the surface of the seeded medium in petriplates. The inoculated plates were kept in the refrigerator at 5^0C for 4 hours to allow the diffusion of chemical into the medium. Then plates were incubated at 30^0C for 72 hours and observed for the production of inhibition zone around the filter paper discs. The results obtained were analysed statistically.

3.4 Evaluation of bioagents

3.4.1 Effect of antagonistic organisms on the growth of *Xanthomonas axonopodis* pv. *punicae* under *In vitro*

Four biocontrol agents *viz., Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Bacillus subtilis* were evaluated for their efficacy against the growth of *X. axonopodis.* pv. *punicae* by inhibition zone assay method. The cultures / formulations of these biocontrol agents were obtained from department of Plant Pathology, University of Agricultural Sciences, Dharwad and Institute of Organic Farming, Dharwad.

A heavy suspension (3 day old) of *X. axonopodis.* pv. *punicae* multiplied in nutrient broth (20 ml) was mixed with lukewarm nutrient agar medium (1000 ml) contained in Erleyenmayer's flask. Fifteen to twenty ml of seeded medium was poured into the sterilized petriplates and allowed to solidify. A loopful culture of each of the antagonistic organism was placed in the centre of petriplates containing the seeded medium. In case of fungal antagonists, mycelial discs of 5 mm (diameter) size taken from actively growing culture were placed in the centre of the plates. The inoculated plates were then incubated at 30^0 C for 72 hours. Observations were recorded for the zone of inhibition produced by antagonistic micro organisms around the growth of the pathogen.

3.4.2 Dual culture plate method:

The molten (45^0C) nutrient agar was poured into sterilized petriplates and allowed to solidify, then the specific antagonist bacteria was streaked across one third of the plate and incubated for 24 hours after which the test bacteria were streaked perpendicular to the zone of inhibition of antagonistic bacteria. The failure of the pathogen to grow near the antagonist colonies indicate the antagonistic activity of the organism (Michael *et al.*, 2000)

3.5 Effect of botanicals

3.5.1 *In vitro* evaluation of plant extracts

Plant based pesticides, which are relatively safe, economical and non-hazardous can be used successfully for the management of bacterial diseases in crop plants. The present investigation is aimed at screening some plant extracts for their antibacterial properties against *X. axonopodis* pv. *punicae*.

The following plant extracts were selected.

Sl. No.	Name of the botanical	Plant part used	Concentration (%)
1.	*Adathoda vasica* (Adathoda)	Leaves and Stem	10,20,30,40
2.	*Annona squamosa* (Custard apple)	Seeds	10,20,30,40
3.	*Alium sativum* (Garlic)	Bulb	10,20,30,40
4.	*Azadirachta indica* (Neem)	Seed oil	10,20,30,40
5.	*Salvadora persica* (Meswak)	Stem	10,20,30,40
6.	*Pogostemon cablin Pellet* (Patchuoli)	Leaves	10,20,30,40
7.	*Ocimum sanctum L.cv. purple* (Tulsi)	Leaves	10,20,30,40

Fresh plant materials were collected and washed first in tap water and then in distilled water, 100 grams of fresh sample was chopped and macerated in a surface sterilized pestle and mortar by adding 100 ml of sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth, filtrate thus obtained was used as a stock solution.

To study the antibacterial mechanism of plant extracts, inhibition zone assay method was followed.

A heavy suspension (72 hours old) of *X. axonopodis.* pv. *punicae*, multiplied in nutrient broth (20 ml) was mixed with molten (50^0C) nutrient agar medium (1000 ml) contained in an Erleyenmayer's flask, so as to get the thick growth of bacteria on the medium, 15 to 20 ml of seeded medium was poured onto the sterilized petriplates and allowed to solidify.

Ten, twenty, thirty and fourty per cent each of plant extract was prepared by mixing 10, 20, 30 and 40 ml of stock solution with 90, 80, 70 and 60 ml of sterilized distilled water, respectively. Filter paper discs (Whatman No. 44) measuring 5 mm diameter were soaked separately in different plant extracts of desired concentrations and then placed onto the surface of the seeded nutrient agar medium. The inoculated plates were first incubated at 5^0C in refrigerator to allow the diffusion of extract into the medium and then incubated at 30^0C for 72 hours.

At the end of incubation period, observations were recorded for the production of inhibition zone representing the efficacy of plant extracts in inhibiting the growth of pathogen. Inhibition zone in each plate was measured in terms of millimetre in diameter and data obtained was analysed statistically.

3.6 Field evaluation of bactericides and antibacterial chemicals against bacterial blight

Chemicals were screened for their efficacy to check the incidence and severity of bacterial blight in pomegranate. This experiment was conducted in Ambiabahar (February – August) of 2010 in farmer field at Bandi village in Yelburga taluq (Koppal-District). The trials were laid out in Randomized Completely Block Design (RCBD) with three replications and there were four plants in each treatment. Variety grown was Kesar. The details of treatments were furnished below.

A total of seven sprays were given at an interval of 15 days with first spray at the disease onset. Spraying was done using manually operated high volume (knapsack) sprayer. Observations on incidence and severity on fruits were recorded before and after third and fifth spray using 0 – 6 scale and per cent disease incidence and per cent disease index was worked out. Fruit yield was recorded and data was analyzed statistically (Sukhatme and Amble, 1985)

3.7 *In vivo* evaluation of bioagents

The field study was taken up to evaluate the different biocontrol agents against the bacterial blight of pomegranate. The experiment was taken up during Ambiabahar (January) cropping season of 2010 in farmers' field at Bandi village (Yalburga taluk). The trial was laid out in randomized completely block design with four replications and there were three plants for each treatment. The bioagents tested are,

1. *Psuedomonas flourescens*
2. *Bacillus subtilis*

The bioagents were sprayed for five times at an interval of 15 days initiating the first spray at the on set of disease on fruits. Observations on the incidence and severity of disease on fruits were recorded before and after third and fifth application of bioagents using 0 – 6 scale. Fruit yield was recorded and analysed statistically.

3.8 Yield parameters

Bahar treatments were imposed during hastabahar season, because of unseasonal rains received during October and November excessive flower drop was observed in the orchard. Further the crop was imposed during ambiabahar. After successive seven sprays of different chemicals/bio agent, the yield contributing parameters like fruit number and average fruit weight was recorded from the all treatments. Further estimated the fruit yield per tree, later it was calculated per hectare yield by taking the count of 645 trees/ha (Spacing of 4.25m x 3.65m).

4. EXPERIMENTAL RESULTS

The results of the investigations undertaken on “Integrated disease management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*” during 2009-10 are presented here under.

4.1 Survey for the incidence and severity of bacterial blight of pomegranate

4.1.1 Incidence and severity of disease in different districts of northern Karnataka

Roving survey was undertaken during 2009-10 to asses the incidence and severity of bacterial blight of pomegranate in major pomegranate growing areas of North Karnataka and results are presented in the Table 1a, b, c, d and e.

Bagalkot district

Survey was conducted in Bagalkot and Mudhol Taluk covering seven villages comprising 21 orchards. Hundred per cent incidence with 50 per cent disease index on fruit was observed at villages Govinakoppa, Kaladagi and Hebbal during mrig bahar. Mean PDI on the leaf was 29.25 on stem 35.57 and on fruits was 35. However, the disease incidence and index on leaf, stem and fruits was minimum at Hebbal village during hastabahar (Table 1a).

Bijapur district

Indi and Bijapur taluk were surveyed covering 23 orchards. Table-1 b indicated that the PDI on leaf, stem and fruit was 29.82, 23.57 and 24.28 respectively. PDI on the fruit was maximum (35%) at Kapnimbargi of Indi taluk and Kanwadi of Bijapur taluk. The PDI on the fruit was minimum (5%) during hastabahar at Jumnal and Utnal villages of Bijapur taluk.

Gadag district

In Gadag district, Gadag and Ron talukas were surveyed covering 7 villages comprising 21 orchards. Results indicate that the mean PDI on leaf, stem and fruit was 32.57 per cent, 28.57 per cent and 25 per cent respectively. PDI on the fruit was observed maximum (40) in Rajore village of Gadag taluk during mrigbahar and minimum (15) during hastbahar at Vajra Bandi village of Ron taluk (Table 1c).

Koppal district

Pomegranate orchards in Gangavathi, Kustagi and Yelburaga taluks were surveyed comprising of seven villages and eighteen orchards. PDI on leaf was 38.22 on stem 30.71 and on fruits it was 29.28. The maximum PDI on the fruit (35) was observed in Bankapur and Kanakgiri village of Gangavati taluk during mrigbahar and ambiabahar and minimum (15), PDI was in Hirehonnapur village of Kustagi taluk during hastabahar (Table 1d).

Raichur District

Seven villages covering 20 orchards were surveyed in Raichur taluk, during ambiabahar, mrigbahar and hastabahar. Maximum PDI on fruits (40%) and on stem (30%) was recorded in Bijangera village during mrigbahar and minimum PDI on fruit 15 was recorded at villages Yapaldinni, Tuntapur, Chandra banda. Mean PDI on the leaf, stem and on fruit was 26.40, 17.85 and 24.28, respectively (Table 1e).

4.1.2 Severity of bacterial blight during different bahars of pomegranate

Survey conducted on severity of bacterial blight of pomegranate in major pomegranate growing districts of North Karnataka, viz: Bagalkot, Bijapur, Gadag, Koppal and Raichur districts during different cropping seasons like mrigbahar, hastabahar and ambibahar revealed that maximum PDI on leaves (43.6%) stem (41.80) and fruit (43.0%) was observed during mrigbahar followed by ambiabahar with PDI of 34.00 on leaves, 26.50 on stem and 29.50 on fruits. The PDI was least during Hastabahar with 17.70 on leaves, 15.30 on stem and 18.20 on fruits.

Table 1a: Survey on the incidence and severity of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in major areas of Northern Karnataka during 2009-10

Sl no.	District	Taluk	Village	No. of orchards	Cropping season	Variety	Leaf		Stem		Fruit	
							Percent Incidence	PDI*	Percent Incidence	PDI*	Percent Incidence	PDI*
1	Bagalkot	Bagalkot	Kaladgi	2	Ambiabahar	Kesar	35	21	80	40	60	30
		Bagalkot	Kaladgi	3	Ambiabahar	Araktha	37	29.6	40	20	60	30
		Mudhol	Govinkoppa	2	Mrigbahar	Sindhoor	80	48	100	50	100	50
		Bagalkot	Kaladgi	3	Mrigbahar	Sindhoor	40	24	90	45	100	50
		Mudhol	Hebbal	4	Mrigbahar	Ganesh	80	48	90	45	100	50
		Mudhol	Mahalingpur	3	Hastabahar	Sindhoor	27	16.2	30	15	40	20
		Mudhol	Hebbal	4	Hastabahar	Kesar	30	18	40	20	30	15
			Total	**21**	**Mean**		**47**	**29.25**	**67.14**	**35.57**	**70**	**35**

* PDI stands for Per cent Disease Index

Table 1b: Survey on the incidence and severity of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in major areas of Northern Karnataka during 2009-10

Sl No.	District	Taluk	Village	No. of orchards	Cropping season	Variety	Leaf		Stem		Fruit	
							Percent Incidence	PDI*	Percent Incidence	PDI*	Percent Incidence	PDI*
1	Bijapur	Bijapur	Kanmadi	3	Ambiabahar	Ganesh	66	39.6	50	25	70	35
		Indi	Atarga	4	Ambiabahar	Araktha	68	40.8	40	20	60	30
		Indi	Sirknalli	2	Mrigbahar	Kesar	63	37.8	80	40	60	30
		Indi	Kapnimbargi	5	Mrigbahar	Ganesh	85	51	80	40	70	35
		Bijapur	Hitnalli	3	Hastabahar	Sindhoor	38	22.8	50	25	60	30
		Bijapur	Jumnal	5	Hastabahar	Ganesh	8	4.8	10	5	10	5
		Bijapur	Utnal	1	Hastabahar	Ganesh	20	12	20	10	10	5
			Total	**23**	**Mean**		**47.71**	**29.82**	**47.14**	**23.57**	**48.57**	**24.28**

* PDI stands for Per cent Disease Index

Table1c: Survey on the incidence and severity of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in major areas of Northern Karnataka during 2009-10

Sl no.	District	Taluk	Village	No. of orchards	Cropping season	Variety	Leaf		Stem		Fruit	
							Percent Incidence	PDI*	Percent Incidence	PDI*	Percent Incidence	PDI*
1	Gadag	Gadag	Musigere	2	Ambiabahar	Ganesh	55	33	30	15	40	20
		Gadag	Gajendragad	4	Ambiabahar	Araktha	65	39	80	40	60	30
		Gadag	Lakkundi	1	Mrigbahar	Kesar	75	45	90	45	50	25
		Gadag	Rajur	2	Mrigbahar	Ganesh	85	51	90	45	80	40
	Gadag	Ron	Vajrabandi	3	Hastabahar	Sindhoor	30	18	40	20	30	15
		Ron	Kotbal	5	Hastabahar	Ganesh	45	27	40	20	50	25
		Ron	Udegere	4	Hastabahar	Ganesh	25	15	30	15	40	20
			Total	**21**	**Mean**		**54.28**	**32.57**	**57.14**	**28.57**	**50**	**25**

* PDI stands for Per cent Disease Index

Table 1d: Survey on the incidence and severity of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in major areas of Northern Karnataka during 2009-10

Sl No.	District	Taluk	Village	No. of orchards	Cropping season	Variety	Leaf		Stem		Fruit	
							Percent Incidence	PDI*	Percent Incidence	PDI*	Percent Incidence	PDI*
1	Koppal	Gangavati	Kanakgiri	2	Ambiabahar	Ganesh	63	37.8	80	40	70	35
			Bandi	3	Ambiabahar	Araktha	73	43.8	40	20	60	30
			Hiremannapur	4	Mrigbahar	Kesar	95	57	100	50	60	30
			Bankapur	2	Mrigbahar	Ganesh	90	54	100	50	70	35
	Koppal	Kustagi	Chumnal	1	Hastabahar	Sindhoor	50	30	30	15	60	30
			Hiremannapur	3	Hastabahar	Ganesh	30	18	40	20	30	15
		Yalburga	Jerkundi	3	Hastabahar	Kesar	45	27	40	20	60	30
			Total	**18**	**Mean**		**63.71**	**38.22**	**61.42**	**30.71**	**58.57**	**29.28**

* PDI stands for Per cent Disease Index

Table 1e: Survey on the incidence and severity of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in major areas of Northern Karnataka during 2009-10

Sl no.	District	Taluk	Village	No. of orchards	Cropping season	Variety	Leaf		Stem		Fruit	
							Percent Incidence	PDI*	Percent Incidence	PDI*	Percent Incidence	PDI*
1	Raichur	Raichur	Chandrabanda	2	Ambiabahar	Ganesh	50	30	50	25	60	30
			Kortakonda	3	Ambiabahar	Araktha	45	27	40	20	50	25
			Bijanagera	2	Mrigbahar	Kesar	70	42	60	30	80	40
			Arkera	1	Mrigbahar	Ganesh	75	45	50	25	60	30
		Raichur	Yappledinni	5	Hastabahar	Sindhoor	20	12	20	10	30	15
			Tuntapur	3	Hastabahar	Ganesh	25	15	20	10	30	15
			Chandrabanda	4	Hastabahar	Kesar	23	13.8	10	5	30	15
			Total	**20**	**Mean**		**44**	**26.4**	**35.71**	**17.85**	**48.57**	**24.28**

* PDI stands for Per cent Disease Index

Table 2: Percent disease index of bacterial blight on leaf, stem and fruit of pomegranate and percent severity on trees during different bahars of 2009-10 in North Karnataka

Sl. No.	District	Mrigbahar				Hastabahar				Ambiabahar			
		Leaves	Stem	Fruit	Per cent Severity on Tree	Leaves	Stem	Fruit	Per cent Severity on Tree	Leaves	Stem	Fruit	Per cent Severity on Tree
1.	Bagalkot	40.0	46.6	50.0	48.3	17.0	17.5	17.5	17.4	25.0	30.0	30.0	29.5
2.	Bijapur	44.5	40.0	65.0	57.9	13.0	13.4	13.4	13.3	39.5	22.5	32.5	31.2
3.	Gadag	48.0	45.0	32.5	36.5	20.0	18.4	20.0	19.6	36.0	27.5	25.0	26.6
4.	Koppal	55.5	50.0	32.5	38.3	25.0	18.9	25.0	23.7	41.0	30.0	32.5	32.8
5.	Raichur	43.5	27.5	35.0	40.0	13.7	8.4	15.0	13.6	28.5	22.5	27.5	26.6
	Mean	**46.3**	**41.8**	**43.0**	**44.2**	**17.7**	**15.3**	**18.2**	**17.5**	**34.0**	**26.5**	**29.5**	**29.3**

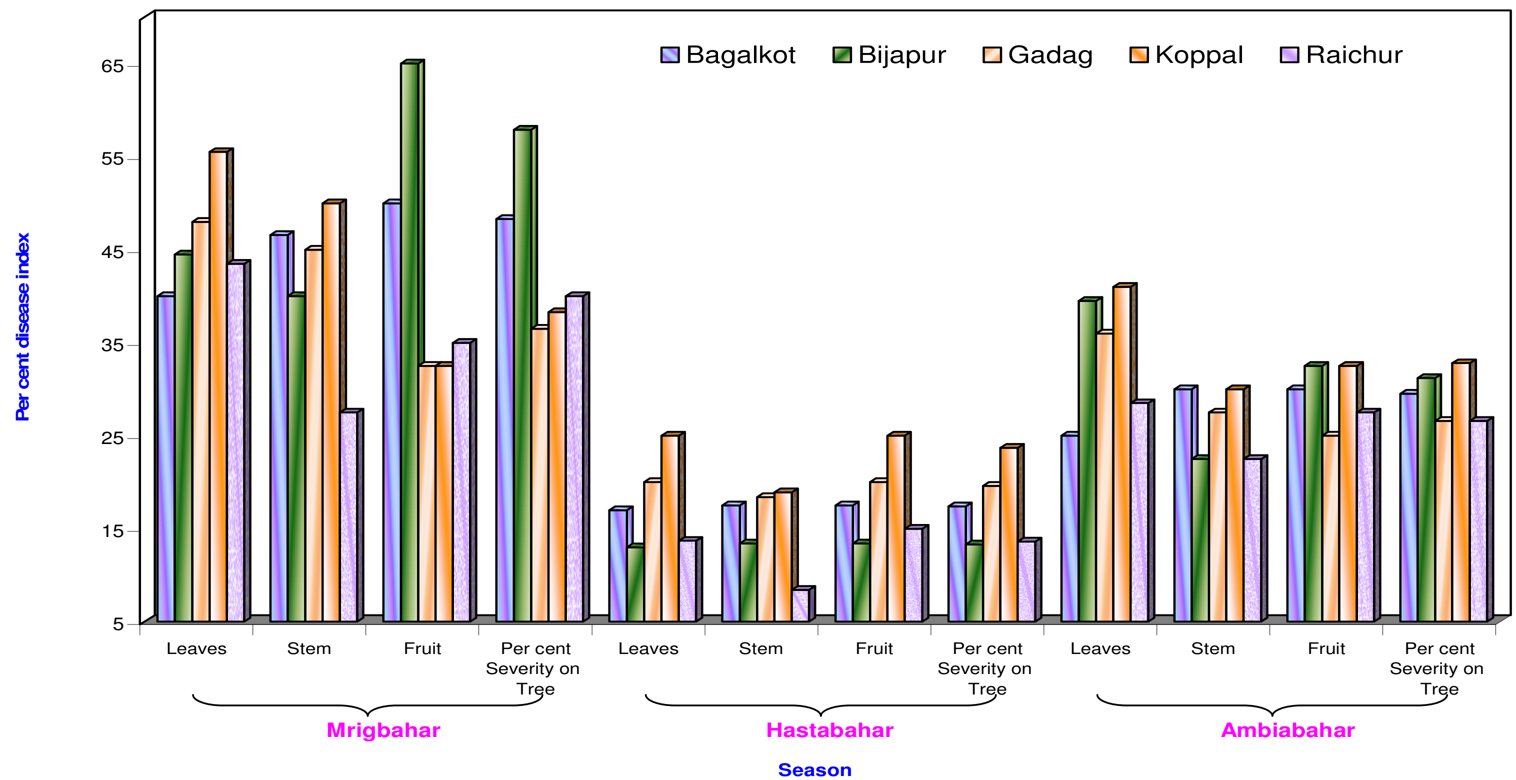


Fig. 1: Per cent disease severity of bacterial blight on leaf, stem and fruit of pomegranate and percent severity on trees during different bahars of 2009-10 in North Karnataka

Fig. 1: Per cent disease severity of bacterial blight on leaf, stem and fruit of pomegranate and percent severity on trees during different bahars of 2009-10 in North Karnataka

Fig. 2: Karnataka map showing surveyed area during 2009-10

Per cent disease severity on the trees was calculated as mentioned in the material and methods. Results revealed that, minimum percent severity on the tree was observed in hastabahar (17.50%) followed by ambiabahar (29.30%) and maximum seventy of 44.20% was recorded in mrigbahar (Table 2).

4.2 Symptomatology

Symptoms were observed on leaf, stem and fruits. Initially small, water soaked, brown to black coloured lesion were noticed on the upper surface of the leaves. Correspondingly on lower surface, diffused water soaked zone was seen around the spot. Spots were round, angular to irregular in shape. As the disease progressed, these spots grew, increased their size (2.0-5.0 mm in diameter), coalesce and extended up to midrib in a week time covering the major portion of the leaf lamina. Severely infected leaves turned yellow, became chlorotic and finally fall off.

Stem infection was manifested in the form of long, narrow and elongated light brown to black coloured lesion (1-4 cm long) on the main stem and branches. The lesions later on became rough and cankerous. As the disease advances, stem girdling and breaking was seen at the point of infection. On developing green fruits, symptoms were noticed as small, pin head sized, black lesions with diffused water soaked margins (oily spots), which later on develops into black coloured, medium to big sized spots (2 to 10 mm in diameter). One to many such spots were seen on the single fruit. Severely infected fruits split opens with L/Y/star shaped cracks within spot. Infected fruits fails to develop, dries up and remain on in the plant until removed manually.

4.3 Isolation of pathogen and maintenance of pure culture

The causal organism *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vauterin *et al.* (1995) was isolated from infected leaf, bark of the stem and pericarp of the fruit showing typical symptoms of bacterial blight. Isolation was done by adapting streak plate method as explained in material and methods. Well separated colonies of each isolate were picked up and streaked on Yeast-Dextrose-Calcium-Carbonate Agar medium (YDCA), incubated at 30^0C for 72 hrs (Plate 2a). The pure colonies obtained were again streaked on Yeast-Dextrose-Calcium-Carbonate Agar (YDCA) slants and kept for incubation at 30^0C for 72 hrs. The cultures so obtained were stored in the refrigerator at 5^0C, which served as a stock culture for further studies. The cultures were renewed by sub-culturing once in a fortnight on YDC Agar slants.

4.4 Proving pathogenicity

Koch's postulates were followed to prove pathogenic nature of *Xanthomonas axonopodis* pv. *punicae* isolates. For proving pathogenicity detached leaf inoculation method was followed (Tuite, 1969). The characteristics symptoms were observed on pomegranate leaves after four days of inoculation as small water soaked lesions. After six days of inoculation it turned brown to black coloured lesions, which later developed into angular to irregular shaped spots along the margins, veins and veinlets of the leaf lamina. Reisolations were carried out from these lesions for each isolate and comparisions were made with original culture to confirm the identity of the pathogen. The reisolated culture resembled the original mother culture and thus pathogenicity test was confirmed.

4.5 *In- vitro* evaluation of chemicals

An investigation was carried out to evaluate commercially available chemicals to find out their effectiveness against the growth of *Xanthomonas axonopodis* pv *punicae* under *in-vitro* condition.

Among different chemicals and their combinations, Streptocycline + Copper oxy chloride had showed highest inhibition (2.8cm) followed by Copper oxy chloride (2.63cm) and Streptocycline (2.59cm), which are on par with each other. All other chemicals *viz*., Streptocycline + Copper hydroxide, Copper hydroxide were found to be moderately effective but were significantly different from each other, Bromapal and Kasagumycin were less effective and were on par with each other.

Twing infection

Stem canker

Infection on leaves

Infection on fruits

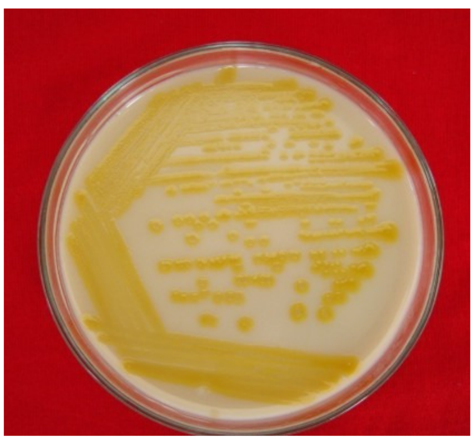
Infection on fruit

Cracking of fruit due to sever infection

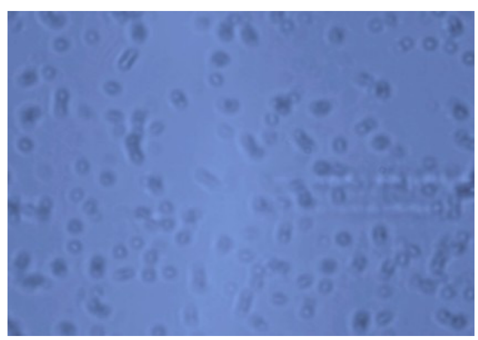
Plate 1: Typical symptoms of bacterial blight on different parts of pomegranate plant

Upper surface of leaves

Lower surface of leaves



Pure culture



Microscopic view 1000X

Plate 2: Pathogencity test

Table 3: *In vitro* evaluation of chemicals against *Xanthomonas axonopodis* pv. *punicae* a causal agent of bacterial blight of pomegranate

Sl. No	Trade name of the chemical	Concentration (ppm)	Mean diameter of inhibition zone (cm)
1.	Bacterinol	250	0.67 (1.29)*
		500	0.72 (1.31)
		750	0.82 (1.34)
2.	Copper oxy chloride	1500	2.62 (1.90)
		2000	2.62 (1.90)
		2500	2.65 (1.91)
3.	Copper hydroxide	1500	1.77 (1.66)
		2000	1.82 (1.67)
		2500	1.82 (1.67)
4.	Kasagumycin	250	0.55 (1.24)
		500	0.55 (1.24)
		750	0.65 (1.28)
5.	Streptocycline	250	2.37 (1.83)
		500	2.60 (1.89)
		750	2.80 (1.94)
6.	Streptocycline + Copper oxy chloride	250 +1500	2.40 (1.84)
		500 +2000	2.75 (1.93)
		750 +2500	3.30 (2.07)
7.	Streptocycline + Copper hydroxide	250 +1500	2.10 (1.76)
		500 +2000	2.17 (1.78)
		750 +2500	2.40 (1.84)
8.	Pathonil	2500	1.15 (1.46)
		5000	1.42 (1.55)
		7500	1.60 (1.61)
9.	Untreated control		0.00 (1.00)
		SE m±	CD at 1%
		0.02	0.08

* Figures in parenthesis are transformed values $\sqrt{x + 1}$

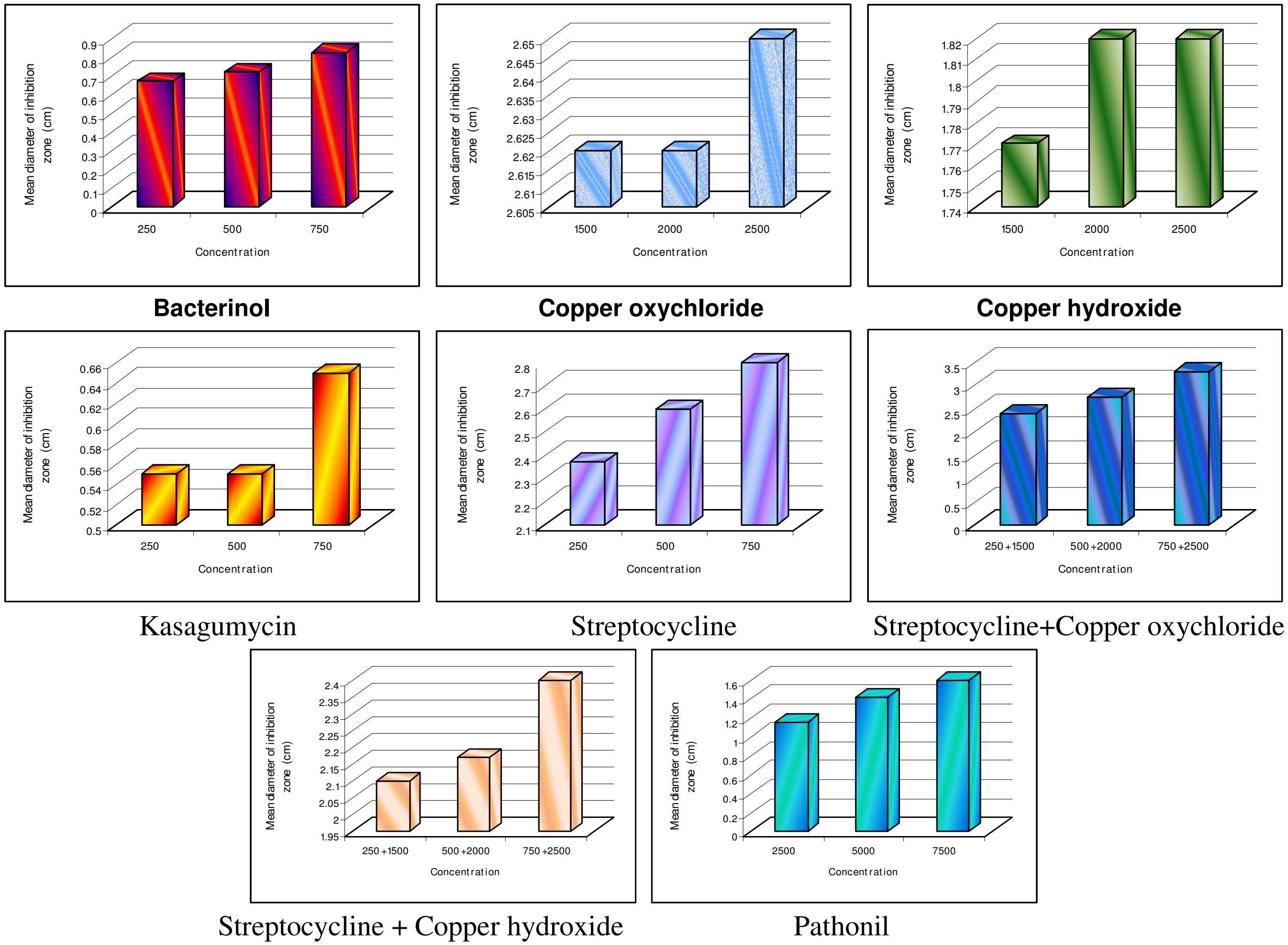
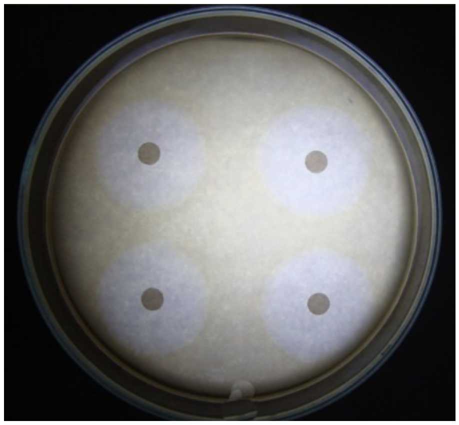
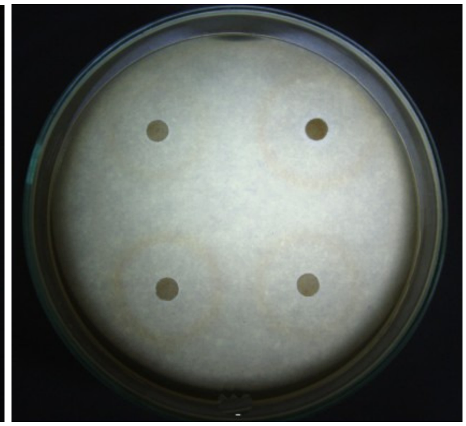


Fig. 3: In vitro evaluation of chemicals against Xanthomonas axonopodis pv. punicae a causal agent of bacterial blight of pomegranate



1



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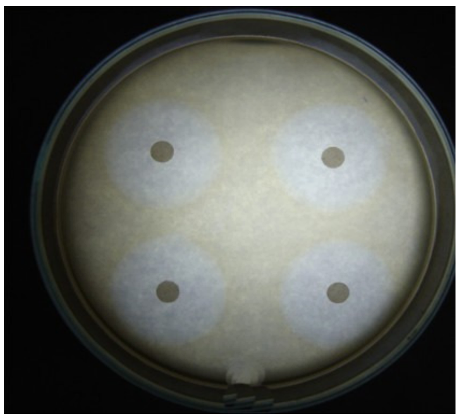

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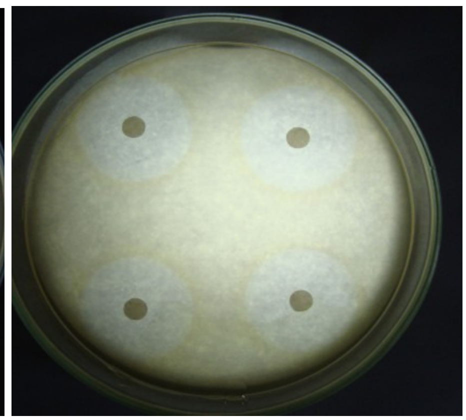

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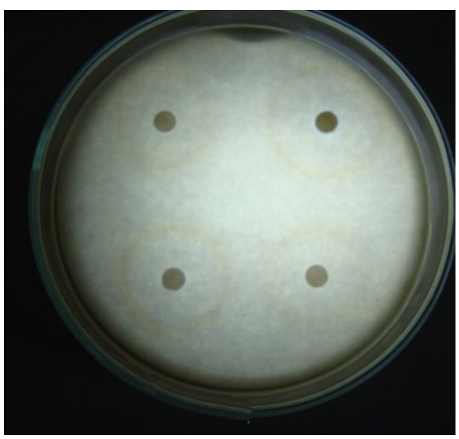

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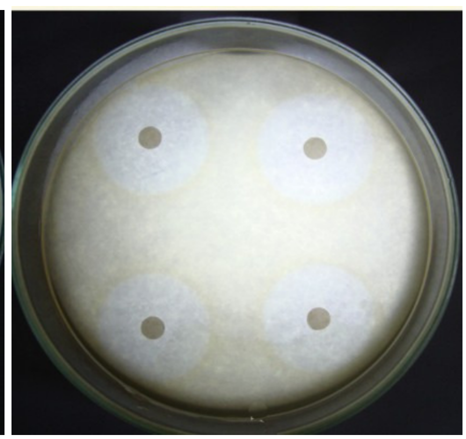


4

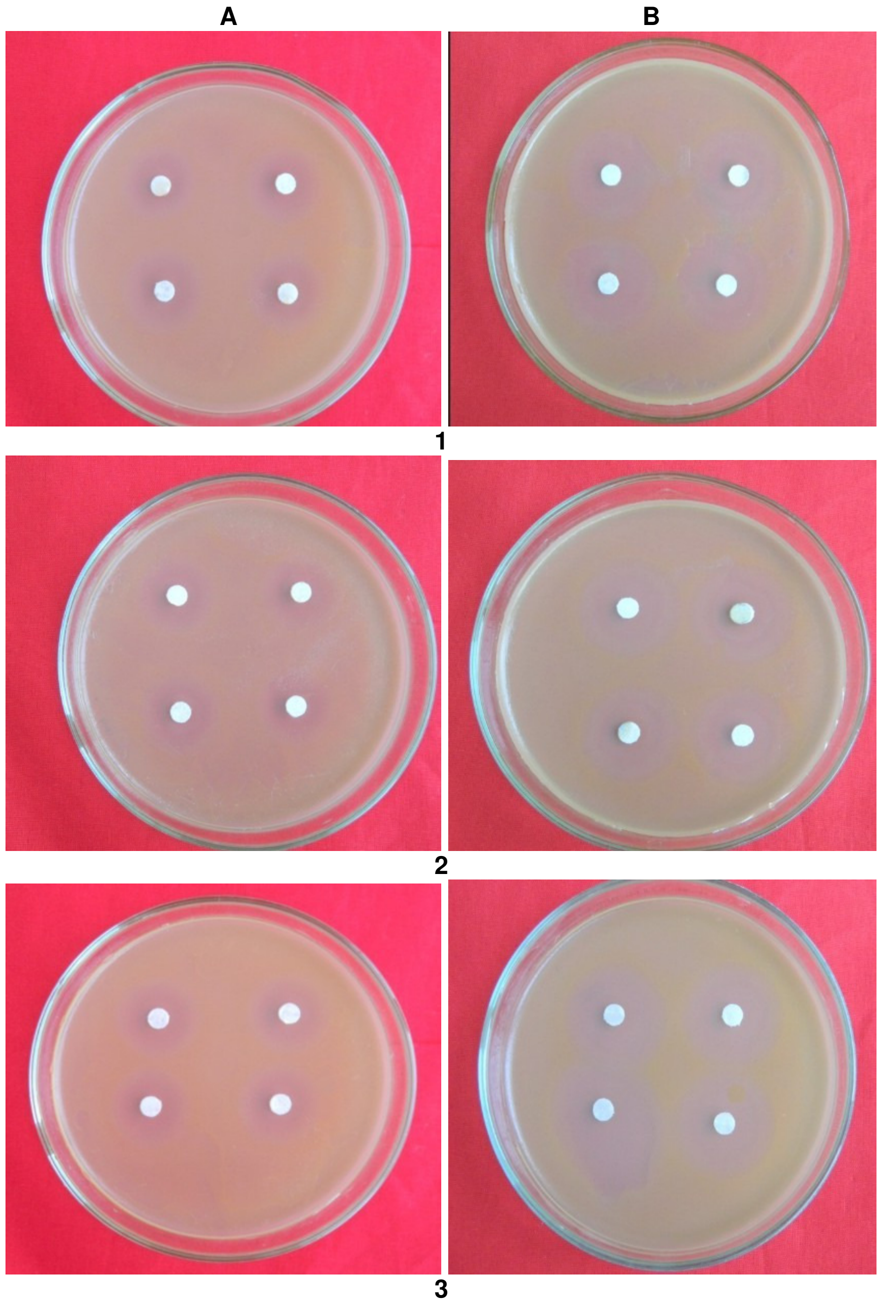
Strepto cycline

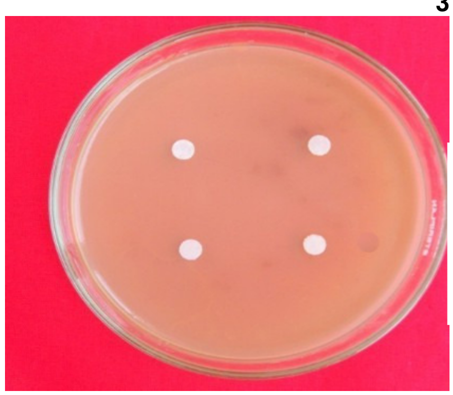
1 – 250 ppm
2 – 500 ppm
3 – 750 ppm

Copper oxychloride

4 – 1500 ppm
5 – 2000 ppm
6 – 2500 ppm

Plate 3: In Vitro evaluation of chemicals





Control

1 – 1500 ppm
2 – 2000 ppm
3 – 2500 ppm
A- Streptocycline + copper hydroxide
B – Streptocycline + copper oxychloride

Plate 4 : In vitro evaluation of chemicals

Between the concentration of each chemicals, efficacy was significant from lower to higher concentration with greater efficacy at higher concentrations.

Interaction effect among the chemicals and concentration indicated that, Streptocycline (750 ppm) + COC (2500 ppm) and Streptocycline at 750 ppm were highly effective with an inhibition zone of 3.3cm and 2.8cm respectively followed by COC 2500 ppm. The moderately effective treatments were Streptocycline (500 ppm) + Copper hydroxide at 2000 ppm (2.4cm), Copper hydroxide at 2000 ppm (1.82), Pathonil at 7500 ppm (1.6cm), Kasagumycin at 7500 ppm (0.65cm), of which Pathonil and Kasagumycin are on par with each other. Bacterinol at 250 ppm (0.67cm) and Kasagumycin at 250 ppm (0.5cm) are also on par with each other.

4.6 *In -vitro* evaluation of botanicals

Out of seven botanicals evaluated against *Xanthomonas axonopodis* pv. *punicae*, Tulsi leaf extract at 40 per cent concentration showed maximum inhibition (1.76cm) followed by Neem seed oil (1.50cm) (Table 4).

Garlic bulb, Meswak stem and leaves of patchouli extracts were found to be on par at 30 per cent concentration. However, custard apple seed extract and leaves and stem extract of adathoda showed no effect on *Xanthomonas axonopodis pv. punicae* at all concentration tested.

Interaction effect among the botanicals and concentration indicated that, tulsi leaves was found to be most effective at 40 per cent with (1.76cm) inhibition zone and the next best botanicals were neem seed oil at 40 per cent concentration (1.50cm) followed by garlic bulb (1.10cm). The moderately effective treatments were patchouli at 40 per cent concentration (0.87cm) and meswak powder at 40% (0.89cm), where as adathoda and custard apple were found in effective.

4.7 Effect of antagonistic organisms on the growth of *Xanthomonas axonopodis* pv.*punicae* under *In -vitro*

Study conducted on effect of antagonistic agent on growth of *Xanthomonas axonopodis* pv.*punicae* (Table-5) indicated that among the four antagonistic agents tested *Pseudomonas flourescens* was found significantly superior in inhibiting the growth of organism (1.77 cm) followed by *Bacillus subtilis* (0.67 cm). However the *Trichoderm viride* and *Trichoderma harzianum* were found ineffective as they failed to inhibit the growth *of Xanthomonas axonopodis* pv. *punicae.*

4.8 Field evaluation

4.8.1 Effect of chemicals/bioagent on disease incidence/severity

This experiment was carried during ambiabahar (January-June, 2010) to test the field efficacy of different commercially available chemicals against the incidence and severity of bacterial blight in pomegranate.

Initially the orchard was affected with bacterial blight with 64.07 per cent incidence. At the end of seventh spray minimum disease was observed in T7 and T8 treatment followed by T3 with values of 24.82, 25.30 and 27.56 per cent respectively. The maximum disease incidence was observed (65.17%) in untreated control (T10) (Table 6).

The per cent disease reduction over control it was maximum 61.91 per cent in T7 followed by in T8 (61.17%). Treatment number 3 and 4 have reduced the disease to the tune of 57.71 and 54.35 per cent respectively.

Table 7 indicated that, before spray PDI on leaves was in the range of 50.12 to 54.56. After 7^{th} spray PDI on leaves significantly reduced in T7 and T8 with 12.56 and 13.30 per cent respectively, followed by T3 treatment (14.42%).

Per cent disease reduction over control after 7th spray was found maximum (79.38%) in T7 followed by (78.16%). The next best treatment is T3 where 76.32% disease reduction was noticed. In untreated control (T10) maximum disease index of 60.92 was recorded from initial disease index of 54.56.

Table 4: ***In vitro*** **evaluation of botanicals against** ***Xanthomonas axonopodis*** **pv.** ***punicae*** **causal agent of bacterial blight of pomegranate**

Sl. No	Name of the botanical	Plant part used	Mean diameter of inhibition zone (cm)			
			Concentration (%)			
			10	20	30	40
1.	*Adathoda vasica* (Adathoda)	Leaves and Stem	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
2.	*Annanosa squamosa* (Custard apple)	Seeds	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
3.	*Alium sativum* (Garlic)	Bulb	0.80 (1.34)	0.82 (1.34)	0.85 (1.36)	1.10 (1.44)
4.	*Azadirachta indica* (Neem)	Seed oil	0.80 (1.34)	1.00 (1.41)	1.22 (1.48)	1.50 (1.58)
5.	*Salvadora persica* (Meswak)	Stem	0.63 (1.27)	0.70 (1.30)	0.84 (1.35)	0.89 (1.37)
6.	*Pogostemon cablin* (Patchuoli*)*	Leaves	0.67 (1.29)	0.72 (1.31)	0.82 (1.34)	0.88 (1.37)
7.	*Ocimum sanctum* (Tulsi)	Leaves	0.70 (1.30)	0.70 (1.30)	1.62 (1.61)	1.76 (1.66)
8.	Control		0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	Factors	SEm±	CD at 1%			
	Botanicals	0.03	0.13			
	Concentration	0.02	0.09			
	Interaction	0.07	0.26			

* Figures in parenthesis are transformed values $\sqrt{x+1}$

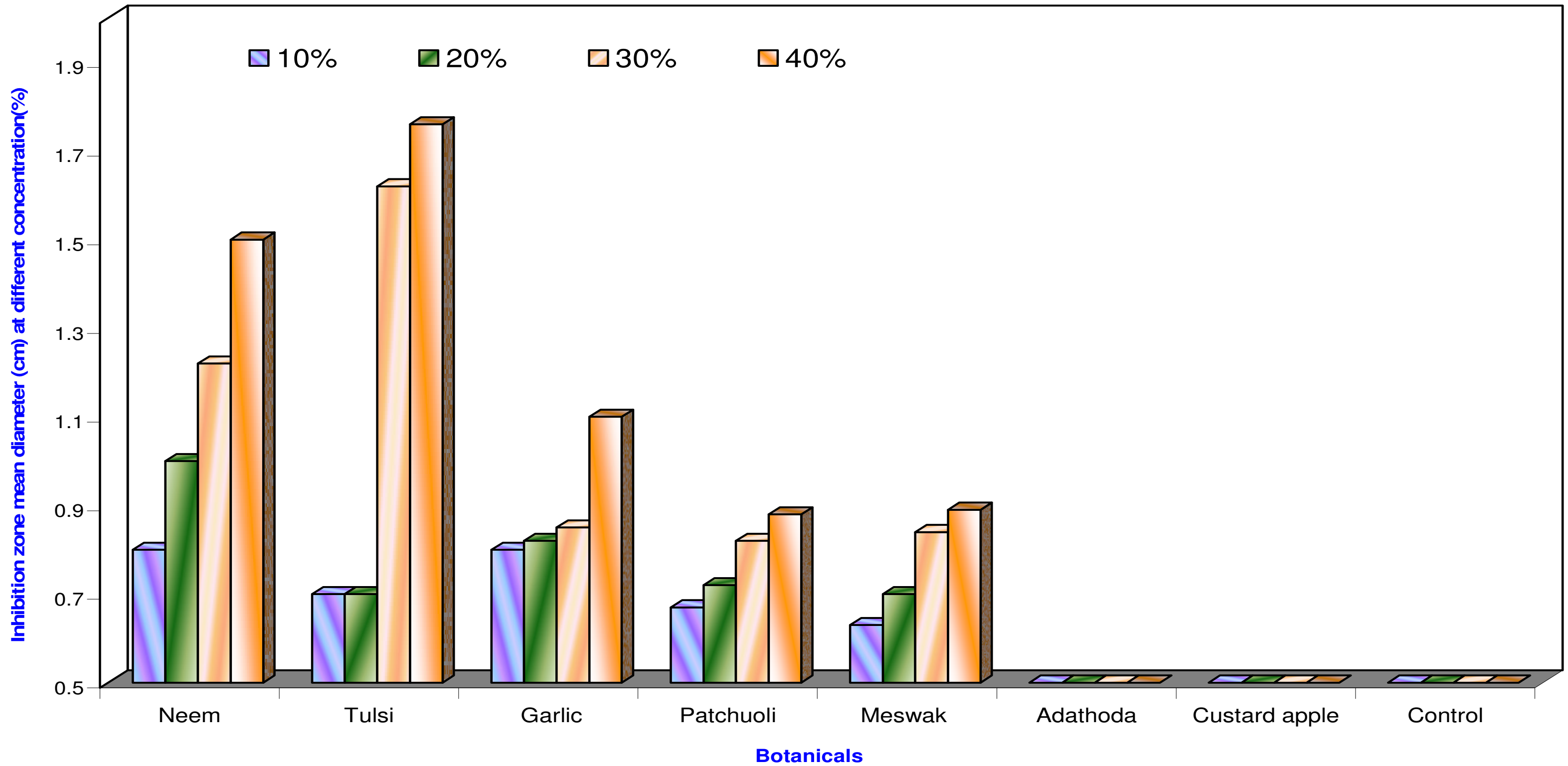
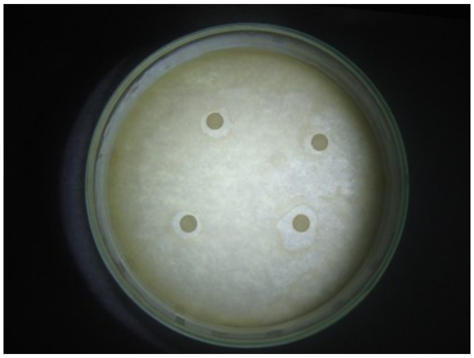


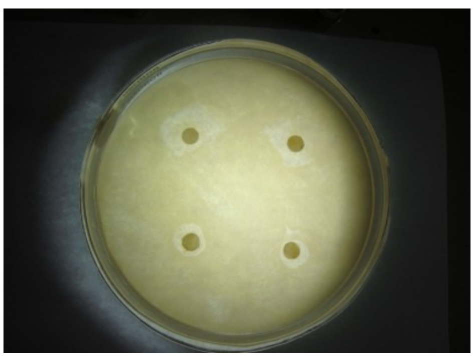
Fig. 4: ***In vitro*** **evaluation of botanicals against *Xanthomonas axonopodis* pv. *punicae* causal agent of bacterial blight of pomegranate**

Fig. 4: In vitro evaluation of botanicals against Xanthomonas axonopodis pv. punicae causal agent of bacterial blight of pomegranate

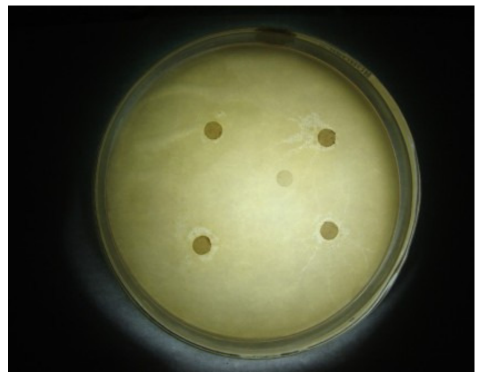


30%

Tulsi leaves



40%



30 %

Neem seed oil

40%

Palte 5 : In vitro evaluation of botanicals

Table 5: *In vitro* evaluation of antagonistic organism against the growth of *Xanthomonas axonopodis* pv. *punicae*

Sl. No	Antagonistic organism	Mean diameter of inhibition zone (cm)
1.	*Bacillus subtilis*	0.67 (1.29)
2.	*Psuedomonas flourescens*	1.77 (1.66)
3.	*Trichoderma viride*	0.00 (1.00)
4.	*Trichoderma harzianum*	0.00 (1.00)
5.	Control	0.00 (1.00)
	SEm±	0.02
	CD at 1%	0.10

* Figures in parenthesis are transformed values $\sqrt{x + 1}$

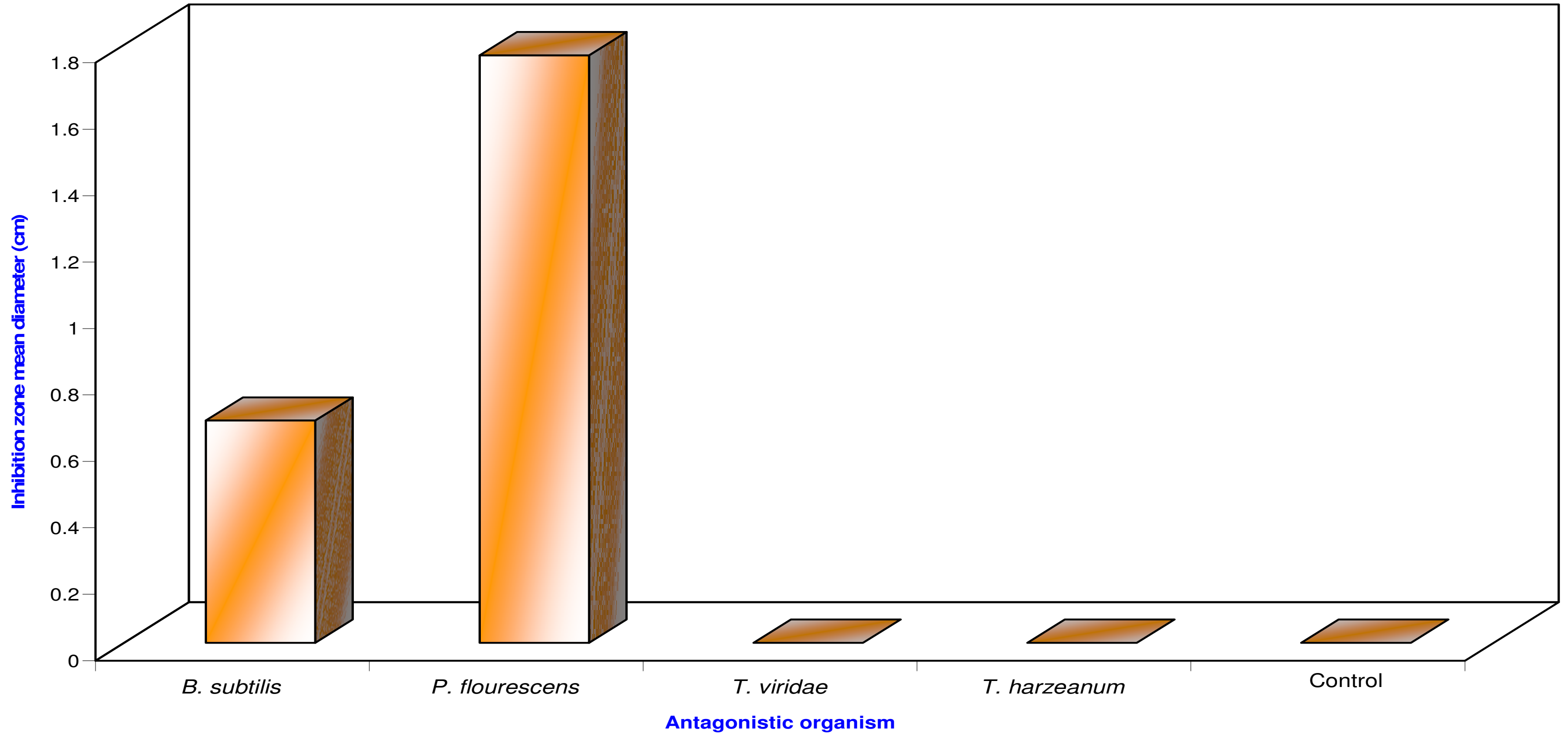
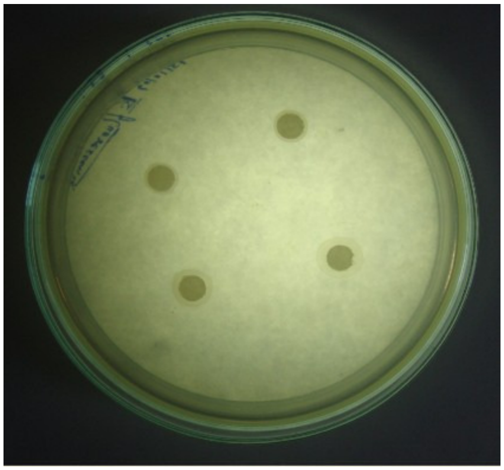
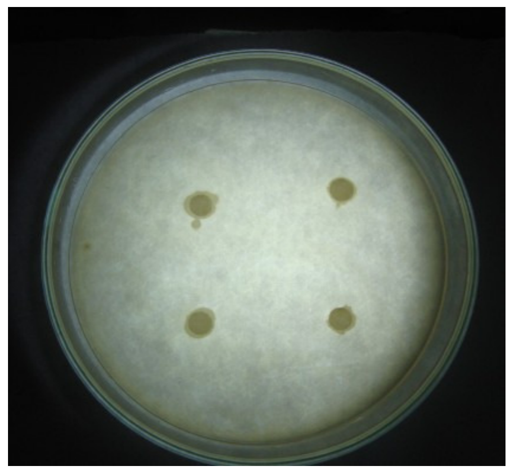


Fig. 5: *In vitro* evaluation of antagonistic organism against the growth of *Xanthomonas axonopodis* pv. *punicae* a causal agent of bacterial blight of pomegranate

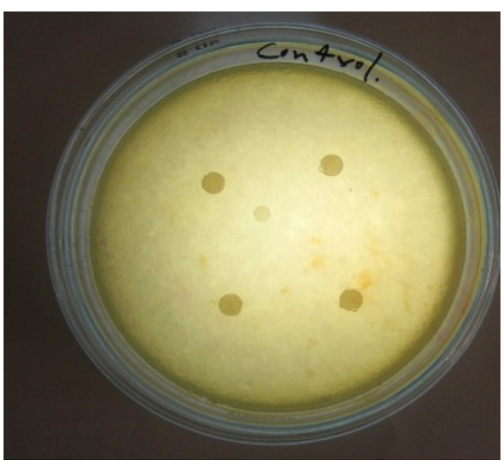
Fig. 5: In vitro evaluation of antagonistic organism against the growth of Xanthomonas axonopodis pv. punicae a causal agent of bacterial blight of pomegranate



Pseudomonas flourescens



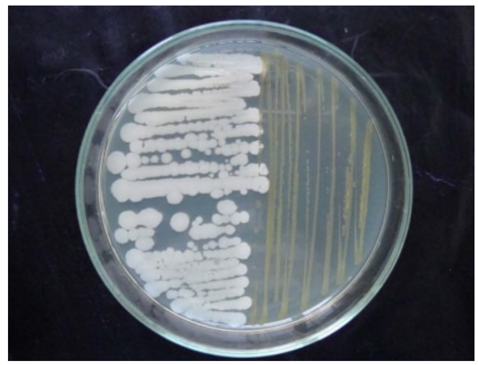
Bacillus subtilis



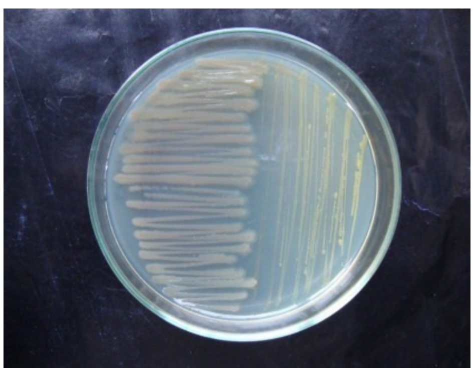
Control

Plate 6: In vitro evaluation of bioagents by inhibition zone assay method

Trichoderma viride



Bacillus subtilis



Pseudomonas flourescens

Trichoderma harzianum

Plate 7: In vitro evaluation of bioagents by dual culture plate method

Table 8 data indicates that, initial percent disease incidence on stem was with a range of 60.23 to 64.66 per cent. At the end of 7th spray, T7 has minimum PDI with an value of 40.59 per cent followed by 46.44 per cent both in T4 and T8 treatments. However, maximum per cent disease incidence on stem was 65.46 per cent in untreated control (T10).

The percent disease reduction over control, it was maximum of 38.00 per cent in T7 followed by T8 and T4 29.05 per cent.

Table 9 indicates that, before spray the initial per cent disease index on stem in the orchard was 45.23 to 49.58 per cent. After 7th spray lowest PDI was observed in T7 treatment (22.60%), which was significant over other treatments. The PDI on stem was maximum (59.26%) in untreated control. The per cent disease reduction over initial disease, was maximum in T7 treatment (61.86%), which was statistically significant.

The per cent disease incidence before spray on fruit was in the range of 38.15 to 41.25 per cent in the orchard(Table.10). After the end of 7th spray per cent disease incidence on the fruit was lowest in T7 (18.52%) and T8 (19.49) followed by T4 (20.54%) and T3 (21.55%). The maximum per cent disease incidence on fruit was observed in untreated control plot (52.22%). Per cent disease reduction over initial disease was maximum in T7 (66.46%) and T8 (64.70%), followed by T4 treatment (62.80%).

Table 11 indicated that, per cent disease index on the fruit was in the range of 24.29 to 32.56 per cent after 3rd spray. In untreated control PDI on fruit was maximum 38.59 per cent. All the treatments except untreated control there was reduction in the disease with a range of 40.52 to 73.69 per cent. At the end of 7th spray, disease has reduced in all the treatment except untreated control. In untreated control, the per cent disease index on fruits has attained maximum of (38.59), it was observed minimum in T7 (10.15) and T8 (11.12) followed by T4 (12.12) and T3 (12.56).

Two bioagents were tested in the field for the management of bacterial blight of pomegranate. Both the bioagents are on par with each other with respect to per cent disease incidence and per cent disease index on leaves (Table 6 and 7).

Disease incidence of 56.65 per cent was recorded in case of *Pseudomonas flourescens* followed by *Bacillus subtilis* 57.26 per cent on stem but they were significantly differ from each other with 36.14 per cent PDI and 38.69 per cent PDI respectively (8 and 9).

Table 10 and 11 indicated that, the disease incidence on fruit 25.00 per cent was recorded in case of *Pseudomonas flourescens* followed by *Bacillus subtilis* 27.92 per cent. Similarly 19.25 per cent and 18.65 per cent of PDI were observed in *Pseudomonas flourescens* and *Bacillus subtilis* treatments respectively and they were significantly differ from each other with respect to per cent disease incidence and per cent disease index on leaves (Table 10 and 11).

4.9 Effect of chemical/ bioagents on the severity of Bacterial blight and yield of pomegranate

Table 12 indicated that, final PDI was maximum (38.59%) in case of control plant where there was no application of chemicals for the management of the disease. The minimum PDI was found in T7 and T8 with 10.15 and 11.12 respectively.

The total number of fruits/plant was in the range of 26.56 to 45.25. Data indicated that the total number of fruits/plant was not affected by the chemicals and bioagents. When we take into consideration healthy fruits/plant it was recorded maximum 42.01 fruits in T7 followed by T8 (38.35). However T4 also found beneficial (34.96).

The total number of healthy fruits/plant was least in untreated trees (8.56). Similarly the average weight of the fruit was found to be maximum in T10 treatment. The fruit yield /tree was maximum of 9.28 kg in T7 followed by 9.01 kg in T8. However, it was least (3.22 kg) in case of untreated control. The estimated yield/ha in tonnes was maximum in T7 treatment followed by T8 treatment with 5.96 and 5.79 tonnes/ha respectively. T3 (5.50 t/ha) and T4 (5.43 t/ha) were also found good with respect to estimated yield/ha. The estimated fruit yield in T10 (untreated control) was minimum (2.07).

Table 6: *In vivo* evaluation of different chemicals/ bioagents/botanical against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv *punicae*: Percent disease incidence on leaves

Treat ments	Chemical name	Trade name	Dosage (%)	Before spray	Percent disease incidence on leaves after 1st to 7th spray							% disease reduction
					1	2	3	4	5	6	7	
T1	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	Streptocycline	0.05	60.12 (50.81)*	59.11 (50.22)	55.28 (48.01)	55.22 (47.97)	48.78 (44.28)	41.97 (40.36)	37.58 (37.79)	29.75 (33.04)	54.35
T2	2-Bromo-2-Nitro Propane-1,3,-Diol	Bacterinol	0.05	62.43 (52.17)	62.11 (51.98)	61.22 (51.46)	60.91 (51.32)	58.19 (49.69)	50.56 (45.30)	39.00 (38.62)	32.99 (35.04)	49.37
T3	Copper oxy chloride	Blitox	0.25	61.42 (51.58)	58.93 (50.12)	56.22 (48.55)	52.24 (46.26)	47.60 (43.60)	41.66 (40.18)	36.49 (37.14)	27.56 (31.65)	57.71
T4	Copper hydroxide	Kocide	0.25	64.07 (53.15)	62.75 (52.36)	62.24 (52.06)	57.64 (49.37)	51.93 (46.08)	43.11 (41.02)	36.86 (37.36)	29.75 (33.04)	54.35
T5	*Pseudomonas fluorescence*	*Pseudomonas fluorescens*	1.00	61.82 (51.81)	61.07 (51.37)	60.52 (51.05)	58.09 (49.63)	55.56 (48.17)	49.10 (44.46)	43.50 (41.24)	32.13 (34.51)	50.69
T6	*Bacillus subtilis*	*Bacillus subtilis*	1.00	60.56 (51.07)	59.16 (50.25)	58.73 (50.00)	54.89 (47.78)	48.29 (44.00)	45.62 (42.47)	41.53 (40.10)	33.95 (35.62)	47.90
T7	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper oxy chloride	Streptocycline+ Blitox	0.05 + 0.25	62.12 (51.99)	61.89 (51.85)	59.72 (50.58)	58.05 (49.61)	51.92 (46.08)	41.97 (40.36)	36.29 (37.02)	24.82 (29.86)	61.91
T8	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper hydroxide	Streptocycline + Kocide	0.05 + 0.25	62.65 (52.30)	58.46 (49.85)	56.03 (48.44)	53.13 (46.77)	48.12 (43.90)	43.11 (41.02)	37.79 (37.91)	25.30 (30.18)	61.17
T9	Quat – NX (Botnical)	Pathonil	0.50	60.58 (51.08)	58.11 (49.64)	58.11 (49.64)	53.80 (47.16)	50.74 (45.40)	47.60 (43.60)	41.81 (40.27)	35.34 (36.46)	45.77
T10	Untreated control			62.05 (51.95)	62.85 (52.42)	63.00 (52.51)	63.24 (52.65)	63.96 (53.08)	64.32 (53.29)	65.14 (53.79)	65.17 (53.80)	
	SEm±			NS	0.38	0.26	0.95	0.23	0.22	0.28	0.51	
	CD at 0.05			NS	1.14	0.80	2.82	0.70	0.67	0.85	1.51	

* Figures in the parenthesis are arc sin transformed values

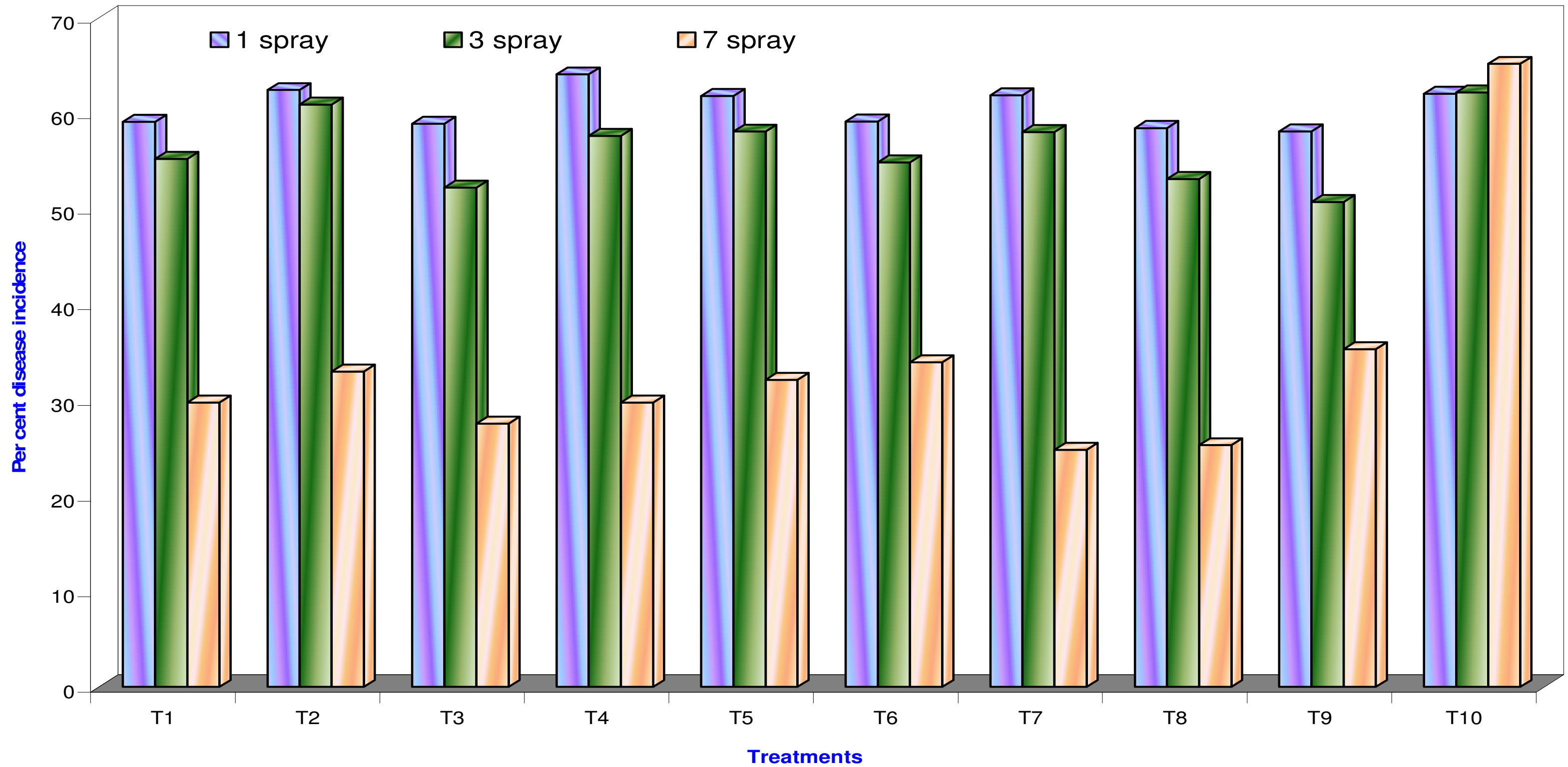


Fig. 6: *In vivo* evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by *Xanthomonas axonpodis* pv *punicae* : per cent disease incidence on leaves

Fig. 6: In vivo evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by Xanthomonas axonpodis pv punicae: per cent disease incidence on leaves

Table 7: *In vivo* evaluation of different chemicals/ bioagents /botanical against bacterial blight of pomegranate caused by *Xanthomonas axonpodis* pv *punicae*: Percent disease index on leaves

Treat ments	Chemical name	Trade name	Dosage (%)	Before spray	Percent disease index on leaves after 1st to 7th spray							% disease reduction
					1	2	3	4	5	6	7	
T1	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	Streptocycline	0.05	53.25 (46.84)*	52.16 (46.21)	48.11 (43.89)	38.12 (38.11)	30.10 (33.26)	28.21 (32.06)	25.44 (30.27)	22.08 (28.01)	63.75
T2	2-Bromo-2-Nitro Propane-1,3,-Diol	Bacterinol	0.05	53.53 (47.00)	51.45 (45.81)	49.59 (44.74)	41.37 (40.01)	36.24 (36.99)	32.90 (34.98)	30.53 (33.52)	23.39 (28.91)	61.60
T3	Copper oxy chloride	Blitox	0.25	54.45 (47.53)	53.12 (46.76)	47.78 (43.66)	38.12 (38.11)	27.26 (31.46)	27.39 (31.34)	25.05 (30.02)	14.42 (22.30)	76.32
T4	Copper hydroxide	Kocide	0.25	54.56 (47.59)	52.95 (46.67)	46.39 (42.91)	33.13 (35.12)	29.76 (33.04)	27.08 (37.22)	24.92 (29.93)	18.45 (25.42)	69.71
T5	*Pseudomonas fluorescence*	*Pseudomonas fluorescence*	1.00	50.12 (45.05)	48.74 (44.26)	45.17 (42.21)	41.20 (39.91)	37.85 (37.95)	36.62 (37.22)	34.22 (35.78)	20.34 (26.72)	66.61
T6	*Bacillus subtilis*	*Bacillus subtilis*	1.00	53.21 (46.82)	51.29 (45.72)	46.40 (42.91)	36.49 (37.14)	32.19 (34.55)	28.46 (32.23)	26.47 (30.95)	20.12 (26.64)	66.97
T7	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper oxy chloride	Streptocycline+ Blitox	0.05 + 0.25	53.45 (46.95)	52.23 (46.25)	47.80 (43.72)	45.60 (42.45)	41.21 (39.92)	31.18 (33.93)	28.45 (32.22)	12.56 (20.74)	79.38
T8	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper hydroxide	Streptocycline + Kocide	0.05 + 0.25	50.23 (45.11)	47.38 (43.48)	38.24 (38.18)	34.05 (35.68)	26.44 (30.93)	22.70 (28.44)	19.82 (26.42)	13.30 (21.38)	78.16
T9	Quat – NX (Botanical))	Pathonil	0.50	53.12 (46.76)	52.75 (46.55)	50.75 (45.41)	46.15 (42.77)	43.10 (41.01)	36.53 (37.17)	31.39 (34.06)	24.56 (29.69)	59.68
T10	Untreated control			53.56 (47.02)	54.21 (47.39)	55.25 (47.99)	56.59 (48.76)	57.25 (49.14)	59.26 (50.31)	60.26 (50.90)	60.92 (51.28)	
	SEm±			NS	0.28	0.28	0.41	0.39	0.28	0.16	0.74	
	CD at 0.05			NS	0.86	0.83	1.23	1.17	0.84	0.49	2.2	

* Figures in the parenthesis are arc sin transformed values

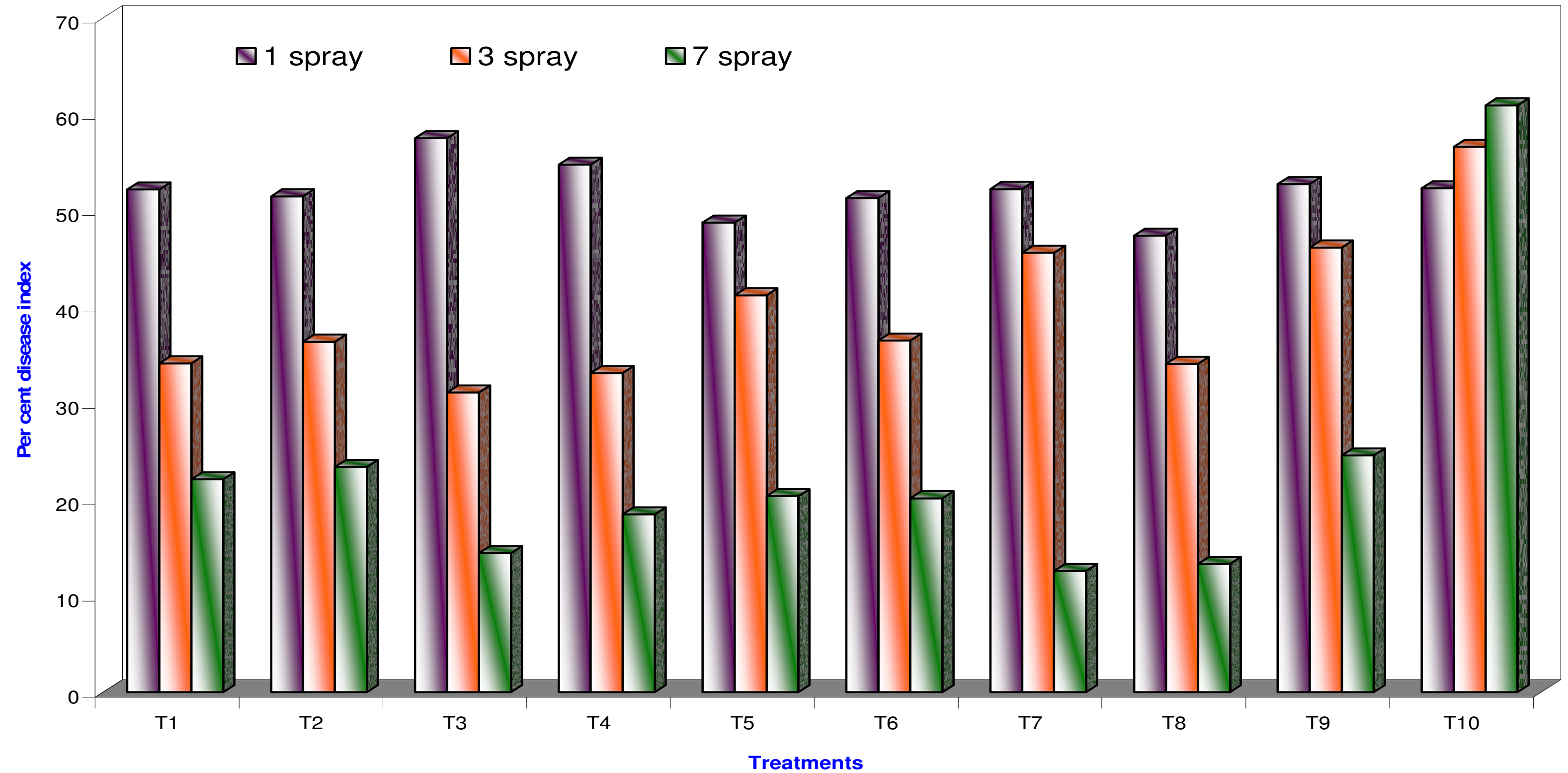


Fig. 7: *In vivo* evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by *Xanthomonas axonpodis* pv *punicae*: per cent disease index on leaves

Fig. 7: In vivo evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by Xanthomonas axonpodis pv punicae: per cent disease index on leaves

Table 8: *In vivo* evaluation of different chemicals/ bioagents/botanical against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv *punicae*: Percent disease incidence on stem

Treatments	Chemical name	Trade name	Dosage (%)	Before spray	Percent disease incidence on stem after 1st to 7th spray							% disease reduction
					1	2	3	4	5	6	7	
T1	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	Streptocycline	0.05	62.53 (52.23)*	62.00 (51.92)	61.18 (51.43)	61.15 (51.42)	60.29 (50.91)	58.14 (49.66)	55.21 (47.97)	51.57 (45.88)	21.21
T2	2-Bromo-2-Nitro Propane-1,3,-Diol	Bacterinol	0.05	63.85 (53.01)	63.25 (52.66)	62.59 (52.27)	61.00 (51.33)	61.99 (51.91)	61.65 (51.71)	59.54 (50.47)	55.90 (48.36)	14.60
T3	Copper oxy chloride	Blitox	0.25	64.66 (53.50)	64.29 (53.28)	64.00 (53.10)	63.92 (53.06)	61.92 (51.87)	60.23 (50.88)	56.25 (48.57)	50.24 (45.11)	23.25
T4	Copper hydroxide	Kocide	0.25	60.60 (51.09)	57.60 (49.35)	57.52 (49.30)	54.64 (47.64)	51.35 (45.75)	51.35 (45.75)	50.16 (45.07)	46.44 (42.94)	29.05
T5	*Pseudomonas fluorescence*	*Pseudomonas fluorescens*	1.00	62.95 (52.48)	61.18 (51.43)	60.54 (51.06)	61.14 (51.41)	61.00 (51.33)	58.65 (49.96)	58.37 (49.79)	56.65 (48.01)	13.45
T6	*Bacillus subtilis*	*Bacillus subtilis*	1.00	62.45 (52.18)	62.00 (51.92)	58.92 (50.11)	58.47 (49.85)	58.30 (49.75)	58.00 (49.58)	57.80 (49.46)	57.26 (49.15)	12.52
T7	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper oxy chloride	Streptocycline+ Blitox	0.05 + 0.25	60.56 (51.07)	58.65 (49.96)	57.22 (49.13)	53.64 (47.06)	49.23 (44.54)	49.23 (44.54)	49.23 (44.54)	40.59 (39.56)	38.00
T8	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper hydroxide	Streptocycline + Kocide	0.05 + 0.25	60.25 (50.89)	57.60 (49.35)	57.52 (49.30)	54.64 (47.64)	51.35 (45.75)	51.35 (45.75)	50.16 (45.07)	46.44 (42.94)	29.05
T9	Quat – NX (Botanical)	Pathonil	0.50	60.23 (50.88)	58.65 (49.96)	57.18 (49.10)	56.25 (48.57)	55.43 (48.09)	55.43 (48.09)	55.45 (48.09)	51.12 (45.62)	22.00
T10	Untreated control			60.58 (51.08)	61.89 (51.85)	62.20 (52.04)	62.34 (52.12)	64.32 (53.29)	64.32 (53.29)	64.49 (53.29)	65.46 (53.98)	
	SEm±			NS	0.22	0.16	0.12	0.14	0.13	0.18	0.25	
	CD at 0.05			NS	0.67	0.49	0.35	0.43	0.41	0.54	0.76	

* Figures in the parenthesis are arc sin transformed values

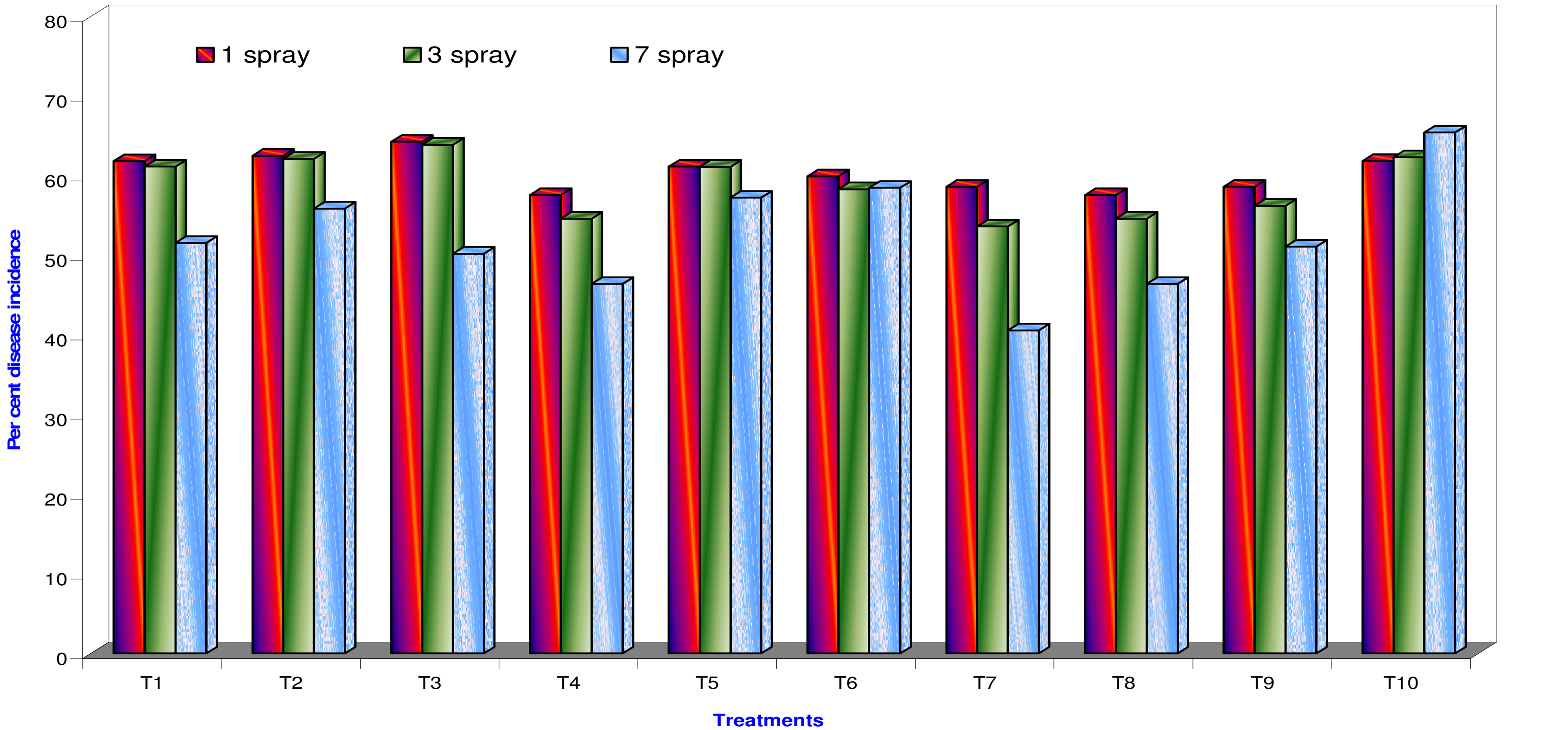


Fig. 8: ***In vivo*** **evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by** ***Xanthomonas xonpodis*** **pv** ***punicae*****: per cent disease incidence on stem**

Fig. 8: In vivo evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by Xanthomonas xonpodis pv punicae: per cent disease incidence on stem

Table 9: *In vivo* evaluation of different chemicals/ bioagents/botanical against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv *punicae*: Percent disease index on stem

Treatments	Chemical name	Trade name	Dosage (%)	Before spray	Percent disease index on stem after 1st to 7th spray							% disease reduction
					1	2	3	4	5	6	7	
T1	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	Streptocycline	0.05	47.78 (43.71)*	46.22 (42.81)	44.95 (42.08)	43.93 (41.49)	42.30 (40.55)	38.81 (38.51)	36.98 (37.43)	32.13 (34.51)	45.78
T2	2-Bromo-2-Nitro Propane-1,3,-Diol	Bacterinol	0.05	47.56 (43.58)	45.62 (42.47)	43.86 (41.45)	40.88 (39.72)	37.84 (37.94)	36.43 (37.11)	34.24 (35.79)	32.43 (34.69)	45.27
T3	Copper oxy chloride	Blitox	0.25	48.36 (44.04)	48.29 (44.00)	46.88 (43.19)	46.00 (42.68)	45.34 (42.30)	43.23 (41.09)	42.08 (40.42)	30.72 (33.64)	48.16
T4	Copper hydroxide	Kocide	0.25	48.25 (43.97)	48.03 (43.85)	46.28 (42.84)	44.63 (41.90)	43.07 (41.00)	41.65 (40.17)	38.98 (38.61)	32.59 (34.79)	45.00
T5	*Pseudomonas fluorescence*	*Pseudomonas fluorescence*	1.00	47.52 (43.56)	46.37 (42.90)	44.61 (41.88)	43.65 (41.35)	41.79 (40.25)	41.45 (40.06)	38.04 (38.06)	36.14 (36.93)	39.01
T6	*Bacillus subtilis*	*Bacillus subtilis*	1.00	47.65 (43.63)	46.30 (42.86)	45.79 (42.56)	43.98 (41.52)	42.31 (40.56)	41.27 (39.95)	39.29 (38.80)	38.69 (38.44)	34.71
T7	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper oxy chloride	Streptocycline+ Blitox	0.05 + 0.25	45.23 (42.24)	44.26 (41.68)	41.91 (40.32)	40.23 (39.35)	39.29 (38.80)	37.97 (38.02)	39.07 (38.67)	22.60 (28.37)	61.86
T8	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper hydroxide	Streptocycline + Kocide	0.05 + 0.25	45.63 (42.47)	45.58 (42.44)	44.21 (41.65)	43.23 (41.09)	39.58 (38.97)	37.23 (37.58)	37.23 (37.58)	31.57 (34.17)	46.72
T9	Quat – NX (Botanical)	Pathonil	0.50	47.58 (43.59)	47.65 (43.63)	46.32 (42.87)	45.06 (42.14)	42.61 (40.73)	40.96 (39.77)	38.31 (38.22)	36.62 (37.22)	38.20
T10	Untreated control			49.58 (44.74)	50.62 (45.33)	50.98 (45.54)	53.92 (47.22)	54.98 (47.83)	56.32 (48.61)	58.45 (49.84)	59.26 (50.31)	
	SEm±				0.22	0.28	0.35	0.27	0.39	0.17	0.67	
	CD at 0.05				0.65	0.85	1.06	0.80	1.18	1.62	1.99	

* Figures in the parenthesis are arc sin transformed values

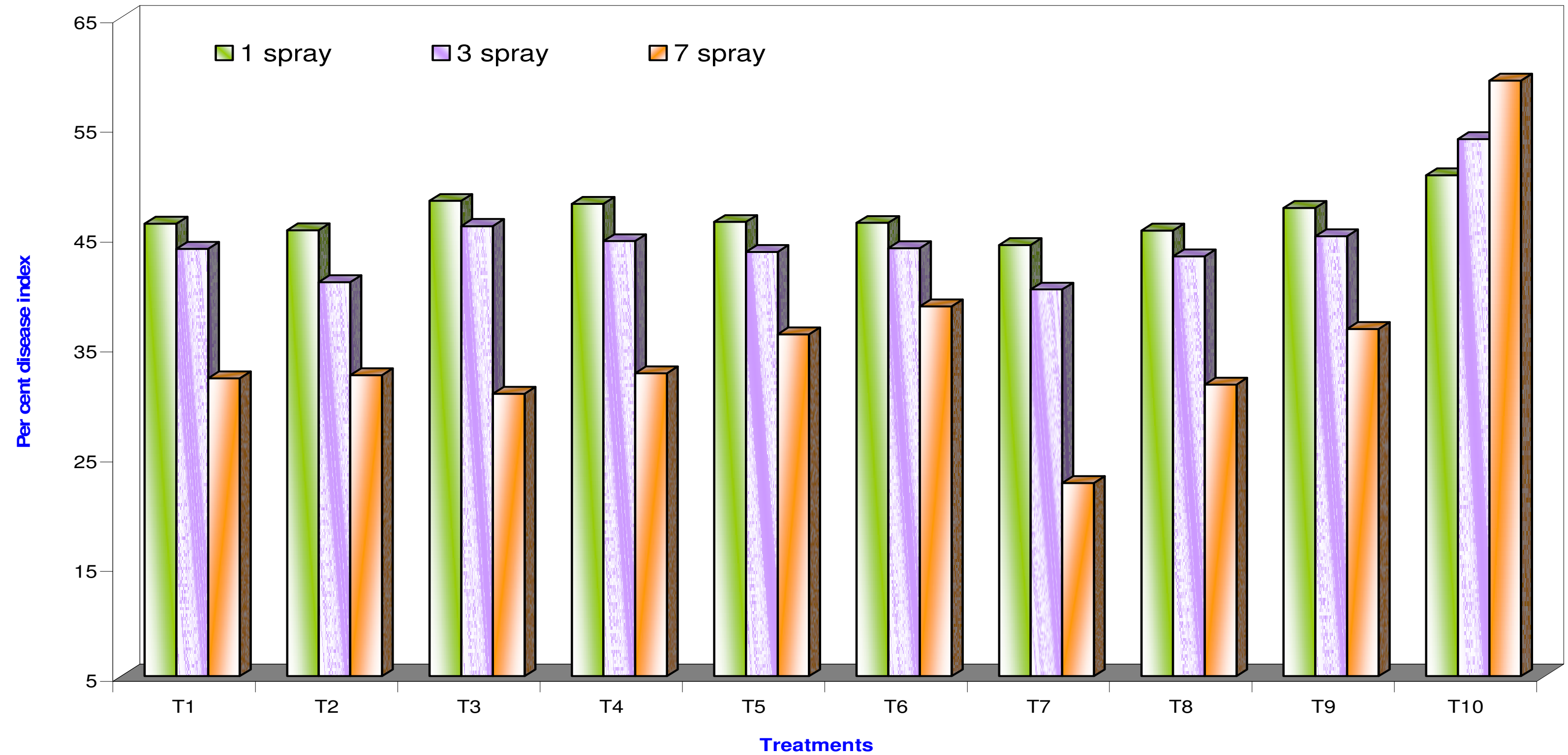


Fig. 9: ***In vivo*** **evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by** ***Xanthomonas axonpodis*** **pv** ***punicae*** **: per cent disease index on stem**

Fig. 9: In vivo evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by Xanthomonas axonpodis pv punicae: per cent disease index on stem

Table 10: *In vivo* evaluation of different chemicals/ bioagents/botanical against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv *punicae*: Percent disease incidence on fruits

Treatments	Chemical name	Trade name	Dosage (%)	Before spray	Percent disease incidence on fruit after 3rd to 7th spray					% disease reduction
					3	4	5	6	7	
T1	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	Streptocycline	0.05	38.25 (38.18)*	37.45 (37.71)	35.87 (36.77)	33.87 (35.57)	30.15 (33.29)	22.23 (28.11)	59.74
T2	2-Bromo-2-Nitro Propane-1,3,-Diol	Bacterinol	0.05	40.52 (39.51)	39.25 (38.77)	35.90 (36.79)	30.87 (33.73)	26.95 (31.26)	26.00 (30.66)	52.91
T3	Copper oxy chloride	Blitox	0.25	38.25 (38.18)	36.52 (37.16)	34.59 (36.01)	32.19 (34.55)	26.91 (31.23)	21.55 (27.65)	60.97
T4	Copper hydroxide	Kocide	0.25	41.25 (39.94)	40.59 (39.56)	36.28 (37.02)	32.83 (34.94)	26.05 (30.67)	20.54 (26.93)	62.80
T5	*Pseudomonas fluorescence*	*Pseudomonas fluorescence*	1	38.15 (38.12)	37.45 (37.71)	34.56 (35.99)	32.15 (34.32)	27.90 (31.87)	25.00 (29.98)	54.72
T6	*Bacillus subtilis*	*Bacillus subtilis*	1	38.66 (38.43)	38.11 (38.10)	37.91 (37.98)	35.83 (36.75)	31.93 (34.39)	27.92 (31.88)	49.43
T7	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper oxy chloride	Streptocycline+ Blitox	0.05 + 0.25	38.45 (38.30)	37.12 (37.52)	36.01 (36.86)	34.24 (35.79)	24.66 (29.76)	18.52 (25.47)	66.46
T8	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper hydroxide	Streptocycline + Kocide	0.05 + 0.25	41.58 (40.13)	39.57 (38.96)	38.26 (38.19)	32.99 (35.04)	26.01 (30.65)	19.49 (26.18)	64.70
T9	Quat – NX (Botanical)	Pathonil	0.5	39.12 (38.70)	38.78 (38.50)	37.95 (38.01)	32.28 (34.60)	27.88 (31.85)	24.88 (29.90)	54.94
T10	Untreated control			39.12 (38.70)	40.22 (39.34)	40.89 (39.73)	42.27 (40.53)	46.23 (42.82)	55.22 (47.97)	
	SEm±			NS	0.21	0.19	0.25	0.17	0.24	
	CD at 0.05			NS	0.64	0.57	0.74	0.52	0.72	

* Figures in the parenthesis are arc sin transformed values

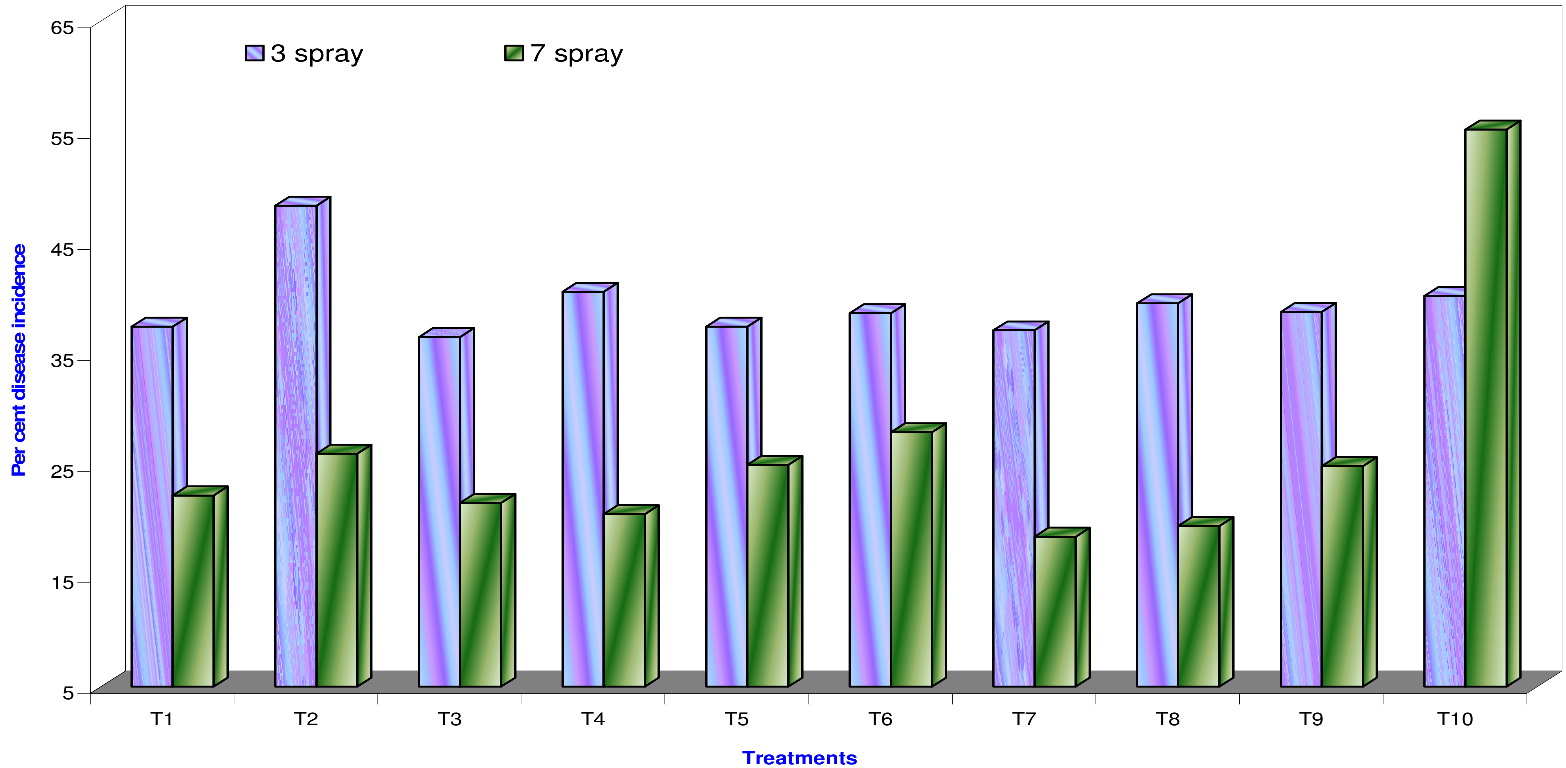


Fig. 10: *In vivo* evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by *Xanthomonas axonpodis* pv *punicae* : per cent disease incidence on fruits

Fig. 10: In vivo evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by Xanthomonas axonpodis pv punicae : per cent disease incidence on fruits

Table 11: *In vivo* evaluation of different chemicals/ bioagents/botanical against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv *punicae*: Percent disease index on fruits

Treatments	Chemical name	Trade name	Dosage (%)	Before spray	Percent disease index on fruits after 3rd to 7th spray					% disease reduction
					3	4	5	6	7	
T1	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	Streptocycline	0.05	29.23 (32.71)*	28.86 (32.48)	22.89 (28.57)	21.91 (27.89)	20.95 (27.22)	13.13 (21.23)	65.97
T2	2-Bromo-2-Nitro Propane-1,3,-Diol	Bacterinol	0.05	32.56 (34.77)	32.00 (34.43)	30.54 (33.53)	28.18 (32.04)	23.25 (28.81)	19.33 (26.07)	49.90
T3	Copper oxy chloride	Blitox	0.25	27.89 (31.86)	26.38 (30.89)	25.34 (30.21)	23.76 (29.16)	21.30 (27.47)	12.56 (20.74)	67.45
T4	Copper hydroxide	Kocide	0.25	26.34 (30.86)	24.90 (29.92)	22.53 (28.32)	20.68 (27.03)	19.62 (26.28)	12.12 (20.36)	68.59
T5	*Pseudomonas fluorescence*	*Pseudomonas fluorescence*	1.00	24.29 (29.15)	24.58 (29.70)	21.95 (27.92)	21.23 (27.42)	20.84 (27.15)	19.25 (26.01)	50.11
T6	*Bacillus subtilis*	*Bacillus subtilis*	1.00	25.69 (30.44)	25.57 (30.36)	21.29 (27.46)	21.59 (27.67)	20.63 (27.00)	18.65 (25.57)	51.67
T7	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper oxy chloride	Streptocycline+ Blitox	0.05 + 0.25	26.54 (30.99)	25.21 (30.12)	23.98 (29.30)	22.33 (28.18)	20.18 (26.68)	10.15 (18.57)	73.69
T8	Streptomycin sulphate 90% , Tetracycline hydroxide 10% + Copper hydroxide	Streptocycline + Kocide	0.05 + 0.25	26.78 (31.15)	25.59 (30.37)	21.92 (27.90)	21.18 (27.39)	20.17 (26.68)	11.12 (19.47)	71.18
T9	Quat – NX (Botanical)	Pathonil	0.50	32.25 (34.59)	31.52 (34.14)	30.00 (33.19)	25.65 (30.41)	24.15 (29.42)	22.95 (28.61)	40.52
T10	Untreated control			25.47 (30.29)	26.06 (30.68)	28.82 (32.45)	30.57 (33.55)	34.51 (35.96)	38.59 (38.38)	
	SEm±			NS	0.21	0.19	0.25	0.17	0.24	
	CD at 0.05			NS	0.64	0.57	0.74	0.52	0.72	

* Figures in the parenthesis are arc sin transformed values

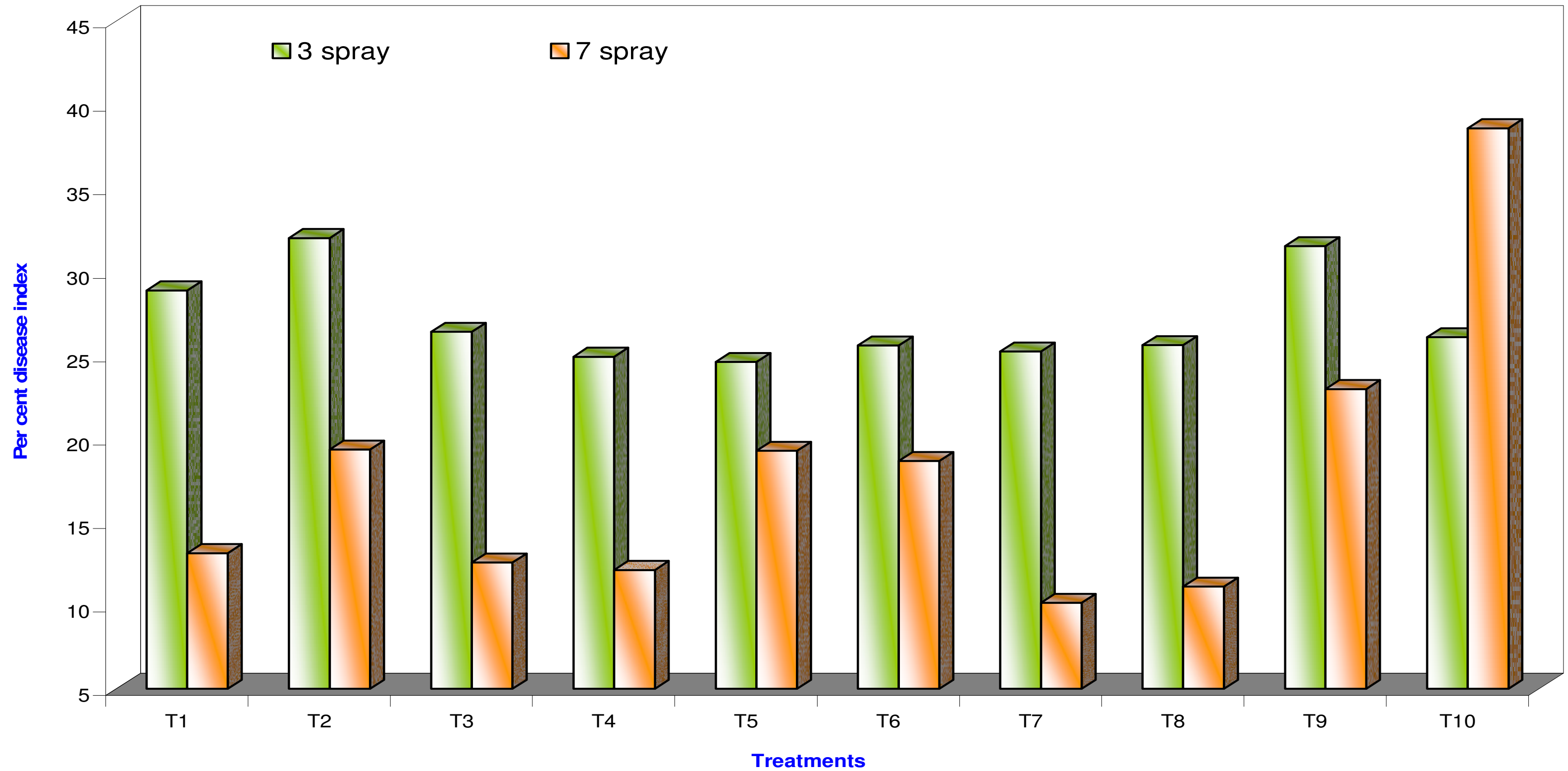


Fig. 11: *In vivo* evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by *Xanthomonas axonpodis* pv *punicae*: per cent disease index on fruits

Fig. 11: In vivo evaluation of different chemicals/ bioagents/ botanicals against bacterial blight of pomegranate caused by Xanthomonas axonpodis pv punicae: per cent disease index on fruits

Table 12: Management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*; fruit yield

Sl. No.	Treatments	Per cent disease index on fruit after 7th spray	Total number of fruits/plant	Total number of healthy fruits/plant	Average weight of the fruit (g)	Fruit yield/ tree (kg)	Estimated yield (tonnes/ha)
1	Streptomycin sulphate 90% + Tetracycline hydroxide 10%	13.13	39.25	31.71	254.78	8.07	5.19
2	2-Bromo-2-Nitro Propane-1,3,-Diol	19.33	38.85	29.64	257.40	7.62	4.90
3	Copper oxy chloride	12.56	40.21	33.98	248.69	8.45	5.43
4	Copper hydroxide	12.12	40.85	34.96	244.79	8.55	5.50
5	*Pseudomonas fluorescence*	19.25	38.45	30.45	260.07	7.91	5.09
6	*Bacillus subtilis*	18.65	37.54	28.62	266.38	7.62	4.90
7	Streptomycin sulphate 90%, Tetracycline hydroxide 10% + Copper oxy chloride	10.15	45.25	42.01	221.00	9.28	5.96
8	Streptomycin sulphate 90%, Tetracycline hydroxide 10% + Copper hydroxide	11.12	42.56	38.35	234.96	9.01	5.79
9	Quat – NX (Botnical)	22.95	37.56	27.61	266.24	7.35	4.72
10	Untreated control	38.59	26.56	8.56	376.50	3.22	2.07
	SEm ±	0.24	NS	0.44	0.29	0.26	0.32
	CD at 5%	0.72	NS	1.25	0.83	0.73	0.92

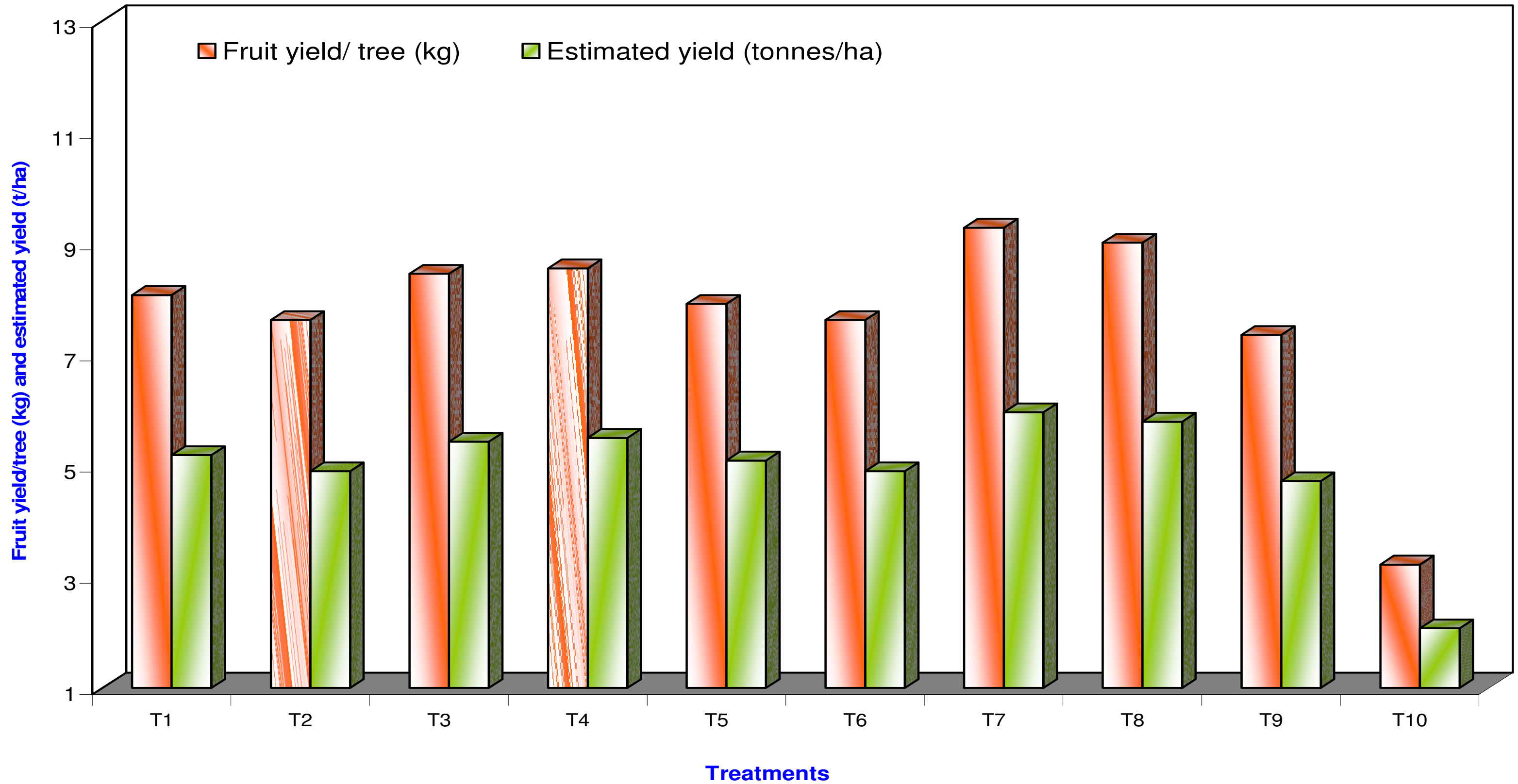


Fig. 12: Management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*; fruit yield

Fig. 12: Management of bacterial blight of pomegranate caused by Xanthomonas axonopodis pv. punicae; fruit yield

Plate 8: In vitro evaluation of chemicals: T7 – Streptocycline + copper oxy

Plate 9: In vitro evaluation of chemicals : T_{10} - Control

5. DISCUSSION

Pomegranate (*Punica granatum* L.), is regarded as a fruit of paradise, is one among the major fruit crops of arid zone. The fruits are immensely important as they are delicious, have high food value and medicinal properties, rich in many nutrients and vitamins. Hence, they are consumed by many people of the world.

Pomegranate suffers from many diseases, among these bacterial blight is major one. Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh, 1959) Vauterin *et al*. (1995) is known to occur in different pomegranate growing areas of the country *viz*., Maharashtra, Karnataka and Andhra Pradesh. The wider range of climatic and environmental conditions in which pomegranate is grown indicates the nature and the diversity of the associated disease problems. It was a dollar earning and booming commercial fruit crop to the farmers until 2002. Later the disease posed its severe out break in all the pomegranate growing regions of Maharashtra, Karnataka, and Andhra Pradesh, which resulted in yield loss both in quality and quantity.

Pomegranate, the boon commercial fruit crop to the farmer, turned as a big bane after the severe incidence of the disease occurred. The disease continued to damage the crop for the subsequent years till date. Although, the farmers have adopted all the available and possible protection measures, the disease could not be managed effectively due to faster inoculum build up and spread. Many growers in the affected areas have uprooted their crop owing to unbearable losses.

For the first time in India, bacterial leaf spot disease of pomegranate was recorded by Hingorani and Mehta (1952), Later during 1959, Hingorani and Singh thoroughly investigated the problem and reported its occurrence in different parts of the country.

Rangaswamy (1962) observed the bacterial leaf spot of pomegranate in Annamalainagar of the then Madras state. The disease was reported by Sohi *et al.* (1964) in Solan region of Himachal Pradesh.

5.1 Survey of bacterial blight of pomegranate

Survey on the incidence and severity of bacterial blight of pomegranate revealed the magnitude of the problem on hand and serves as a precursor for evolving the management strategies. Sudarshan Rao (1975) stated that, survey form the basis for any successful plant protection that depends on early detection of the disease followed by timely adoption of control measures. Hence, in the present investigation, roving survey was undertaken for two years in major pomegranate growing areas of Karnataka to assess the incidence and severity of bacterial blight. During the survey, it was generally observed that, disease incidence on fruit was more than its severity in most of the areas surveyed.

From the survey data, it was observed that, fruits were more vulnerable to the bacterium than leaf as evidenced by more disease incidence and severity on fruits, irrespective of season, location and variety. Among the different districts surveyed maximum disease incidence on fruit (70.00%) was recorded in Bagalkot district followed by Koppal (58.57%), Gadag (50.00%), Bijapur (48.57%) and Raichur (48.57) districts. Correspondingly, average PDI on fruits was observed as maximum in Bagalkot district (35.00%) and minimum was found in Bijapur and Raichr district (24.28%).

Similarly, the PDI on leaf was maximum in Koppal district with 38.22 per cent on the contrary, lowest PDI of 26.40% was recorded in Raichur district.

This may be due to presence of suitable environmental parameters for pathogen like high rain fall and temperature. The disease was comparatively less during hastabahar, this may be due to the presence of unfavorable factors for the bacteria, like lower temperature and dry weather.

Ravikumar *et al.* (2004) in their survey report on bacterial diseases of fruits and vegetables in and around Bagalkot and Bijapur district mentioned 20 to 90 per cent bacterial blight incidence on pomegranate.

Benagi *et al.* (2009) also reported that the severity of disease was more during mrig bahar. He also reported that it was in severe form in the all pomegranate growing areas, which ranged from 0.67-94.80% of severity on a tree. The disease was very severe in Bagalkot district with an average severity value of (74.08%). The minimum severity (6.73%) was observed in Bellary district.

The present findings are also in conformity with the work of Manjula and Khan (2002), who reported the devastating nature of bacterial blight of pomegranate in Bellary, Bijapur and Bagalkot districts on all the varieties, irrespective of age of the plant during late summer and *kharif* season of 2001-02. Among the seasons, mrigbahar was found more vulnerable than ambiabahar and hastbahar because of environmental factors like rainfall and temperature which are less in ambiabahar and hastabahar season.

5.2 Symptomatology

The disease manifested itself on all aerial parts of the plants such as leaf, stem and fruits. Initially, there were small, water soaked lesions on the lower surface of the leaves surrounded by diffused water soaked margin. Correspondingly on the upper leaf surface, small brown to black coloured spots were seen. The spots took the shape of either round or angular to irregular. As the disease progressed, the spots enlarged, (2.0 – 5.0 mm in diameter), coalesced and extended upto midrib in a week time and occupied the major portion of leaf lamina. At later stages, the infected leaves turned yellow, became chlorotic and drop of prematurely. Stem infection was manifested in the form of long, narrow, brown to black coloured lesions (1 – 4 cm long) on the main stem and branches. The lesions later on turned rough and cankerous. Stem girdling followed by breaking at the point of infection was noticed eventually. On flower buds, small water soaked lesions, appeared, which later on turned to brown to black coloured spots leading to dropping of buds under severe incidence. Symptoms on fruits were very conspicuous with small pin head sized lesions surrounded by diffused water soaked margin as a mark of bacterial infection. The lesions later on developed into brownish black coloured, medium to big sized spots (2 – 10 mm in diameter). One to many such spots could be seen on the single fruits. In severe case of infection, the affected fruits split opens with L/Y/ star shaped cracks on the pericarp. Infected fruits did not develop further nor dropped but dried up and remain on the plant.

Similar kind of symptoms on leaf were described by Hingorani and Mehta (1952) as irregular spots varying 2 to 5 mm diameter in size, initially light brown in colour surrounded by a water soaked margin. Later on, spots turned dark brown, coalesced and covered the larger areas of leaf lamina lead to the shedding of leaf at the final stage. They did not notice any symptoms on twigs/branches and fruits.

Kanwar (1976) reported the flower and fruit infection apart from leaf as studied in the present investigation. The symptom similarity on the fruit described by him coincides with present study.

During 1993, Kishun described the bacterial blight symptoms on stem and fruits of pomegranate in a similar way, which were described in the present study.

Further, the bacterial blight symptoms on pomegranate described by various workers from different parts of the country *viz.,* Rangaswamy (1962) from Tamil Nadu, Sohi *et al.* (1964) from Himachal Pradesh, Manjula and Khan (2002) and Yenjerappa *et al.* (2004) from Karnataka were having resemblance with the symptoms studied in the present investigation.

5.3 Isolation and pathogenicity

The causal organism was isolated from the infected leaf, fruit and stem parts by following standard serial dilution plating technique using nutrient agar medium. Repeated isolation of the pathogen showed typical, yellow, mucoid bacterial colonies on nutrient agar medium after 72 hours of incubation at 30^0C. Culture of bacteria was purified by streaking suspected single colony on to the yeast dextrose calcium carbonate agar medium. The pathogen produced pale yellow to dark yellow colonies with mucoid and convex appearance on YDCA medium. Pathogenicity was proved by inoculating bacterial cell suspension (5×10^6 cfu/ml) to the susceptible pomegranate plants of Bhagwa variety. The characteristic symptoms of the disease appeared after 10 days of inoculation in the form of small, water soaked, brown to black coloured lesions, which later on developed into angular to irregular shaped spots along the veins and veinlets of the leaf lamina. Reisolation carried out from artificially inoculated plants yielded the bacterial colonies similar to the original culture.

Hingorani and Mehta (1952) isolated the bacterial pathogen from infected pomegranate leaves and proved pathogenicity. Infection was readily seen by them on tender leaves artificially inoculated plants in seven to ten days of incubation. Isolation and pathogenicity studies were also carried out in a similar fashion by Kanwar (1976). He observed the symptoms within four to seven days on injured portions and it took eight to twelve days to get the symptoms on uninjured parts.

5.4 Evaluation of bactericides/antibiotics

5.4.1 *In vitro* studies

In vitro evaluation of chemicals provides the preliminary information about the efficacy of particular chemical in a shortest period of time and therefore it serves as a basis for further field assay. Chakravarti and Rangarajan (1966) studied the *In vitro* effect of streptocycline on the species of *Xanthomonas*, *Erwinia, Pseudomonas, Corynebacterium* and *Agrobacterium*.

Among the different chemicals evaluated in the present investigation, in inhibiting the growth of *Xanthomonas axonopodis* pv. *punicae* Streptocycline + COC with an inhibition zone of 3.3cm exhibited superior efficacy followed by Streptocycline (2.80cm) and COC (2.65cm). However, both the treatments were on par with each other. All other chemicals *viz.,* Streptocycline + Copper hydroxide, Copper hydroxide were found to be moderately effective but were significantly different from each other, Bromopal and Kasagumycin were less effective and were on par with each other. In respect of concentration, efficacy of each chemical was significant at relatively higher concentrations.

The present findings are in agreement with Sharma *et al.* (1981), who reported that, the combination of streptocycline and copper sulphate was most effective in inhibiting the growth of *Xanthomonas vesicatoria* as assessed by *in vitro* paper disc method.

Venugopal (1983) studied the *in vitro* sensitivity of different isolates of *X. campestris* pv. *mangiferaeindicae* to streptomycin and paushamycin @ 100 and 250 ppm concentrations, respectively.

Manjula *et al.* (2002) also recorded the highest inhibition zone produced by paushamycin (0.05%) and K cycline (0.05%) against the growth of *Xanthomonas axonopodis* pv. *punicae*. Bactrinol (0.05%) and bacteriomycin were the next best effective chemicals and kasugamycin @ 500 ppm concentration was least effective.

5.5 Evaluation of botanicals

5.5.1 *In vitro* evaluation of plant extracts

Botanicals next to bioagents are safe, ecofriendly and cost effective means of managing the crop diseases effectively. In the present study, among the nine different plant extracts screened against *X. axonopodis* pv. *punicae*, none of the plant extracts were found to inhibit the growth of the pathogen completely. However, some of these plant extracts exhibited considerable amount of inhibition.

Among the plant extracts under evaluation, tulsi leaves followed by neem seed oil, garlic bulb extract and patchouli leaves found effective. Tulsi leaves and neem seed oil showed an on par efficacy with each other and were found significantly superior over other treatments in the study irrespective of concentrations. The other plant extracts *viz.,* adathoda leaves and custard apple seeds failed to inhibit the growth of the pathogen even at higher concentration of 40 per cent. Overall effect of plant extracts revealed that, Tulsi leaves at 40% and 30% concentration was significantly greater in efficacy than all other treatments followed by neem seed oil and garlic bulb extract at 40% concentration.

The essential oil from *Ocimum sanctum* leaves showed considerable antibacterial and antifungal activity when tested against six fungal and four bacterial strains. It contains basic active ingredients like methyl cinnamate, citral, eugenol, linalool, camphor and methyl chavicol. This basic active ingredients might have played role in inhibiting the growth of the pathogen *Xanthomonas axonopodis* pv *punicae*. (Dey and Choudhury, 1984)

Mahmoodin, a limonoid, isolated from *Azadirachta indica* oil, showed significant antibacterial activity against various Gram-positive and Gram negative organisms. These compounds might have played role in inhibiting the growth of the pathogen *Xanthomonas axonopodis* pv *punicae*.(Siddiqui *et al.,* 1992)

The antibacterial activity of garlic has been known for a long time. Allicin was initially considered to be the principle, responsible for this activity but it was later proved that allicin is derived from alliin-allinase system present in raw garlic. Presence of this antibacterial compound might be responsible for inhibiting the growth of the pathogen *Xanthomonas axonopodis* pv *punicae*.(Uchida *et al.,* 1975)

Srinivasachary (1995) reported ocimum plant extract as most effective botanical against the growth of *Xanthomonas campestris* pv. *mori* isolated from mulberry. Similar results were found in present investigation. According to Sushma Joseph (1997), garlic extract was highly inhibitory to the growth of *Xanthomonas axonopodis* pv. *dieffenbachiae* followed by ocimum and citronella. The antibacterial properties of onion and garlic were attributed to the presence of sulphur as an active principle (Mangamma and Sreeramulu, 1991).

5.6 Evaluation of bioagents

5.6.1 *In-vitro* effect of antagonistic organisms on the growth of *Xanthomonas axonopodis* pv. *punicae*

In the light of present day constraints with the use of chemical pesticides in plant disease management, the biological control as an alternate option is gaining importance and awareness as the approach is ecofriendly and cost effective. Under biological control of plant diseases, various antagonistic organisms have been identified, which fight against the pathogens by different mechanisms *viz.,* competition, lysis, antibiosis, siderophore production and hyper parasistism (Vidyasekaran, 1999).

Among the different antagonists tried as a biocontrol agents in the present study, *Bacillus subtilis* and *Pseudomonas fluorescens* were found significantly superior over other antagonists in inhibiting the growth of the pathogen. The fungal biocontrol agents *viz., Trichoderma viridae* and *Trichoderma harzianum* were found totally ineffective.

This findings were earlier confirmed by Unnamalai and Gnanamanickam (1984) reported the inhibiting effect of *Pseudomonas fluorescens* on the growth of *Xanthomonas citri*.

Antagonistic activity of *Erwinia herbicola* and *Bacillus subtilis* against *Xanthomonas campestris* pv. *viticola* was observed by Chand *et al*. (1991).

Laha *et al.* (1992) stated that fluorescent pigments produced by *Pseudomonas* are sequester Fe^{3-} ions (Ferrric) and are termed as siderophores, which act as a inhibitors for the growth of some phytopathogenic bacteria and fungi. Biochemical studies conducted by Valasubramanian *et al.* (1994) showed that efficient strains of *Pseudomonas fluorescens* produces an antibiotic phenazine-1-carboxylic acid (PCA) responsible for hindering the growth of plant pathogenic bacteria.

5.7 *In-vivo* evaluation of bactericides and antibacterial chemicals against bacterial blight of pomegranate

The study was conducted during ambiabahar to assess the efficacy of commercially available chemicals for the management of bacterial blight of pomegranate.

Results of ambiabahar season trial indicated that, Streptocycline + COC (0.05%+0.25%) and Streptocycline + Copper hydroxide (0.05%+0.25%) were significantly effective than all other chemicals in minimizing the disease incidence followed by Copper hydroxide, COC each at 0.25%, Streptocycline at 0.05%, Pathonil at 0.5% and Bromopal at 0.05%.

In respect of reducing the disease severity, all the chemicals were found to be significantly effective with the record of lowest disease severity ranged between 12.56 to 24.56 PDI on leaves, 22.60 to 38.69 PDI on stem and 10.15 to 22.95 PDI on fruits.

Streptocycline and COC was found to be most effective because of synergetic effect in which copper will helps to lysis of cell wall. The enzymes which require free sulphydral groups for activity appear to be especially susceptible to inactivation by ions of heavy metals. It is therefore quite possible that copper kills spores by combining with the sulphhydral groups of certain enzymes (Nene and Thapliyal, 2002)

Kishun and Sohi (1984) reported that black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris* was effectively controlled by spray application of agrimycin-100 (0.1%) or Streptocycline @ 250 ppm. Present findings also indicated the efficacy of Streptocycline in reducing the disease severity.

The present findings on efficacy of Streptocycline or other bactericides *viz.,* Bactrinashak or bactinash-200 were in accordance with the results obtained by Manjula *et al.* (2002) as they achieved the effective control of bacterial blight of pomegranate with the sprays of Streptocycline or K cycline or bacterinol-100.

In general, disease severity recorded in all the treated plots was correspondingly lower than the incidence irrespective of cropping season and stage of observation. However, after fifth application of different treatments, average disease severity recorded was significantly low in all the chemicals treated plots Streptocycline + COC (0.05%+0.25%) treatment indicating greater efficacy of all the bactericides.

5.8 *In-vivo* evaluation of antagonistic organisms against the bacterial blight of pomegranate

The two bio agents *Pseudomonas fluorescence* at 1.00 per cent and *Bacillus subtilis* at 1.00 per cent showed moderate effective and they are significantly different from each other.

The presence of fluorescent pigments produced by *Pseudomonas* are sequester Fe^{3-} and are termed as siderophores, which act as a inhibitors for the growth of some phytopathogenic bacteria may be responsible for inhibiting the growth of *Xanthomonas axonopodis* pv. *punicae.*

The efficacy of *Pseudomonas fluorescens* in reducing the bacterial blight intensity on rice was reported by Kaur and Thind (2002).

Lodha (2001) studied the effect of application of two phylloplane antagonist, a white sterile fungus and *Bacillus subtilis* against bacterial blight of cluster bean and the author emphasized the superior efficacy of white sterile fungus followed by *Bacillus subtilis* in reducing the bacterial blight severity of cluster bean. Characterization and evaluation of native strains of rice associated Bacillus strains on rice that substantially suppressed the bacterial blight and increasing the tiller number and grain yield were described by Vasudevan and Gnanamanickam (2002). The results of present investigation derive support from the findings of Dutta *et al.* (2005), who reported that among the 21 rhizobacterial and 52 phylloplane bacterial isolates of mungbean, the isolates MRb (*Pseudomonas fluorescens*) and Plb-3 (*Bacillus* spp.) were effective in reducing the seedling and trifoliate leaf infection of mungbean induced *X. axonopodis* pv. *vignaradiatae*

5.9 Effect of chemicals/ bioagents on severity of bacterial blight and yield of pomegranate

Data on yield parameter revealed that, highest fruit yield of 6.12 tonnes per ha and 5.91 tonnes per ha was recorded in Streptocycline + COC treated plot, followed by Streptocycline + Kocide in ambiabahar cropping seasons respectively as influenced by effective disease control. Similar results obtained by Manjula *et al.* (2002) as they achieved the effective control of bacterial blight of pomegranate with the sprays of Streptocycline or K cycline or bacterinol-100. The technical grade of bactrinol-100 is same as that of bronip/bactrinashak/bactinash-200 *i.e.,* 2 bromo-2nitro propane, 1, 3, diol.

Yenjerappa *et al.* (2004) reported the superior efficacy of Streptocycline (0.05%) in combination with Copper oxychloride (0.2%) in mitigating the bacterial blight menace on pomegranate. In the present investigation, also it was found that treatments, Streptocycline + COC and Streptoclycine + Copper hydroxide were found superior in managing the bacterial blight disease.

Further the findings are in line with findings of Ravikumar *et al.*(2002) reported that least bacterial leaf spot incidence on grape vine was recorded in the plots treated with Streptocycline or Streptomycin sulphate each at 0.05% concentration.

6. SUMMARY AND CONCLUSIONS

Pomegranate (*Punica granatum* L.) is an important fruit crop in India and in subtropical countries of the world. This is subjected to various disease problems and bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh, 1959) Vauterin *et al*. (1995) is one of the important diseases, which caused a great havoc in recent years.

The present investigation was carried out during 2009 - 2010 in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad.

The farmers of Maharashtra and Karnataka, whoever cultivating this crop, they were always on profitable side. Since 2002, the growers were in dire straits due to severe outbreak of bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae*. The disease posed its severity in all the pomegranate growing regions of Maharashtra, Karnataka and Andhra Pradesh resulting huge losses both in terms of quality and quantity. Although, the farmers have adopted heavy chemical protection, which all went in vain due to fast inoculum buildup and wide spread of the disease.

Considering the magnitude of the disease and its resultant losses, the investigation was undertaken to study the disease and pathogen thoroughly and to bring out an appropriate management aspects to mitigate the problem effectively.

Survey conducted during 2009-10 revealed the highest disease incidence on fruits in the villages of Bagalkot district followed by Koppal and Gadag districts. Correspondingly, highest disease intensity on both leaf and fruit was recorded in Bagalkot district. Lowest disease incidence and severity was observed in the villages of Raichur district and disease was severe in mrigbahar cropping season compared to hastabahar and ambiabahar season.

The causal organism was isolated from the infected leaf, fruit and stem parts by following the serial dilution plating technique using nutrient agar medium. Culture of each isolate was purified by streaking suspected single colony on the yeast dextrose calcium carbonate agar.

Pathogenic test revealed that the *X. axonopodis* pv*. punicae* was pathogenic to pomegranate and produced typical symptoms on pomegranate leaves after four days of inoculation as small water soaked lesions. After six days of inoculation it turned brown to black coloured lesions, which later developed into angular to irregular spots along the margins, veins and veinlets of leaf lamina.

Among the different chemicals evaluated to prevent the spread of disease from stem infection revealed that, COC and Copper hydroxide (0.25%) each in combination with Streptocycline (0.05%) recorded the least disease incidence and severity. Similarly, COC (0.25%) and Streptocycline (0.05%), Copper hydroxide (0.25%) were found significantly effective in reducing the disease severity.

In vitro evaluation of chemicals indicated that, COC + Streptocycline with an inhibition zone of 3.3cm exhibited superior efficacy followed by COC (2.63cm) and Streptocycline (2.59cm). Whereas in field evaluation, Streptocycline (0.05%) + COC (0.2%) was found highly effective in reducing the disease incidence followed by Streptocycline (0.05%) + Copper hydroxide (0.25%) , Copper hydroxide, COC each at 0.25%, Streptocycline at 0.05%, Pathonil at 0.5% and Bacterinol at 0.05%. In respect of reducing disease severity, all these bactericides were found equally and significantly effective.

Among biocontrol agents, *Bacillus subtilis* and *Pseudomonas fluorescens* were significantly superior to other antagonists in inhibiting the growth of the pathogen. In field trial, they found moderately effective at 1% concentration.

In vitro evaluation of different plant extracts revealed that, Tulsi leaves at 40% produced the maximum inhibition zone followed by neem seed oil at 30%, garlic bulb extract at 10% and patchouli leaves at 40% concentration.

The highest yield was recorded in Streptocycline + COC and followed by Streptocycline + Copper hydroxide treatment 6.12 and 5.91 tonnes/ha respectively.

Conclusion

1. From the survey results the Percent disease incidence and Percent disease index was found maximum on fruit in the areas of Bagalkot district.
2. It was found that fruits are more vulnerable to the disease than the leaves and stem.
3. Streptocycline in combination with COC was found to be the most effective in controlling the pathogen both under *in-vitro* and *in-vivo* conditions.
4. Among the bioagents *Pseudomonas fluorescence* and *Bacillus subtilis* were found to be moderately effective.
5. Among the botanicals Tulsi leaves followed by Neem seed oil were found superior in inhibiting the pathogen growth under *in-vitro* compared to other botanicals.

Future line of work

1. Management of bacterial blight of pomegranate through transgenics needs to be studied.
2. Need to develop resistant varities against bacterial blight
3. Epidemiological studies to be studied with respect to with spread and survival of pathogen and also role weather parameter to develop modules.
4. Detail research need to be concentrated on role of botanicals and bioagents in managing the disease.
5. There is also an urgent need to develop integrated disease management practices in order to save the crop.

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MANAGEMENT OF BACTERIAL BLIGHT OF POMEGRANATE CAUSED BY *Xanthomonas axonopodis* pv*. punicae* (Hingorani and Singh) Vauterin *et al.*

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ABSTRACT

Considering the magnitude of the disease and its resultant losses, the investigation was undertaken to study the disease and pathogen thoroughly and to bring out an appropriate management aspects to mitigate the problem effectively. Survey conducted during 2009-10 revealed the highest disease incidence (70%) and severity (35%) on fruits in the villages of Bagalkot district followed by Koppal and Gadag districts. Lowest disease incidence (48.57 %) and severity (24.28%) was observed in the villages of Raichur district. It was severe in mrigbahar cropping season compared to hastabahar and ambiabahar. The causal organism was isolated from the infected leaf, fruit and stem parts by following the serial dilution plating technique using nutrient agar medium. Pathogenic test revealed that the *Xanthomonas axonopodis* pv*. punicae* was pathogenic to pomegranate and produced typical symptoms on pomegranate leaves after four days of inoculation as small water soaked lesions. *In vitro* evaluation of chemicals indicated that, Streptocycline (0.05%) + COC (0.2%) exhibited superior efficacy followed by Streptocycline (0.05%) + Copper hydroxide (0.2%) than COC and Streptocycline alone. Among bioagents *Pseudomonas fluorescens* was superior in inhibiting growth of bacteria followed by *Bacillus subtilis.* Whereas, Trichoderma sp. were found ineffective. Among plant extracts tulsi leaves and neem seed oil at higher concentrations (30 and 40 %) were found inhibitory. *In vivo* studies indicated that Streptocycline (0.05%) + COC (0.2%) and Streptocycline (0.05%) + Copper hydroxide (0.2%) found effective in reducing the disease severity of 73.7 and 71.2 per cent respectively. 0.2 per cent copper hydroxide (68.59 %) and copper oxy chloride (67.45 %) also reduced the disease. At 1 per cent *Bacillus subtilis* (51.67 %) and *Pseudomonas fluorescens* (50.11%) also found moderate effective.