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

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1. INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to family Punicaceae is an ancient fruit crop of India. It is native to Iran but extensively cultivated in Mediterranean regions specially in Spain, Morocco, Egypt and Afghanistan. It is also grown in Burma, China, Japan, USA, USSR, Bulgaria and Southern Italy. It is regarded as "vital cash crop" of an Indian farmer and is grown in an area of 1.3 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 of total area in the country followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. Karnataka has the distinction of cultivating pomegranate under tropical conditions with an area of 12727 ha spread across different districts Bijapur, Bellary, Koppal, Bagalkot, Chitradurga, Belgaum, Tumkur, Kolar and Bangalore etc. The production is 1,24,389 tones (Anon., 2005a).

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, excellent keeping quality and remunerative prices in domestic as well as export markets. It thrives well in dry tropics and sub-tropics and comes up very well in soils of low fertility status, adding to that it is salt tolerant too.

The fruit has a wide consumer preference for its attractive, juicy, sweet, acidic and refreshing arils. There is a growing demand for good quality fruits both for fresh use and processing into juice, syrup and wine. 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.

Pomegranate is good source of carbohydrates and minerals such as calcium, iron and sulphur. It is rich in vitamin C and citric acid is the 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 possess pharmaceutical and therapeutic properties. Sweet varieties are mildly laxative, sour types are good against inflammation of stomach and heartache. Flower buds are very useful in Ayurveda for managing bronchitis. The bark (stem) and rind (fruit) portion is used to treat diarrhea 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).

Pomegranate production is also associated with many problems like other crops. Inherent constraints are long dry spells, non-availability of suitable varieties, environmental vagaries, nutritional deficiencies, physiological disorders, post-harvest glut, post-harvest losses, improper storage and lack of marketing facilities, price fluctuation. Biotic constraints are pest and disease problems.

Among these several constraints, losses due to pests and diseases are very high. Although, 25 to 30 per cent of total cost of production is being spent on plant protection specially pesticides, the biotic constraints could not be managed effectively. Among the diseases infecting pomegranate, the bacterial disease popularly known as 'bacterial blight' caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vauterin *et al.* is as a major production constraint.

Chand and Kishun (1991) noticed the epidemics of bacterial blight of pomegranate causing 60 to 80 per cent losses at Indian Institute of Horticultural Research (IIHR) experimental plots. They isolated the causal organism and identified it as *Xanthomonas campestris* pv. *punicae* based on its pathological, cultural, biochemical and physiological features. Later on during 1995, Vauterin *et al.* named the causal organism as *Xanthomonas axonopodis* pv. *punicae* depending on the presence or absence of metabolic activity on different carbon substrates.

Ever since the farmers of Maharashtra and Karnataka who ever cultivating pomegranate, they were always on profitable side from Rs. 60,000 to Rs. 1,00,000 per ha, but since 2002, the growers are in dire straits due to the severe out break of bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae*. The disease, which was of minor importance earlier appeared as a serious threat in all the pomegranate growing regions of Northern Karnataka (Anon., 2002) and Yenjeerappa *et al.* (2004), Maharashtra and Andhra Pradesh resulting in huge yield losses both in terms of quality and quantity. The disease continued to damage the crop (for subsequent years uptill now), although farmers have adopted all possible and available protection measures, the disease could not be mitigated effectively due to rapid build up of inoculum and wide spread of the disease. Pomegranate "the boon commercial fruit crop to the farmer turned as a big bane after the severe out break of bacterial blight. Many growers finding no options to mitigate the disease effectively have uprooted their crop owing to unbearable losses.

For the successful management of any disease under normal conditions, clean sanitation, eradiation of primary source and chemical protection at initial stages are some of the measures recommended. However, these measures are not enough, whenever the outbreak of disease occurred. Hence, thorough understanding of the disease epidemiology and concrete package is necessary to address the menace effectively, so as to save the crop at large.

Now, it is learnt that, bacterial blight of pomegranate is wide spread and is a major production constraint. The disease prevailed in all the seasons with varying degree of severity. Systematic survey for the incidence and severity of the disease in different growing regions is essential to design the appropriate management strategy. Hence, in the present investigation, it was intended to take up fixed plot survey in major affected areas of northern Karnataka and adjoining villages of Andhra Pradesh to generate the information on disease prevalence along with its intensity.

Studies on cultural, morphological, physiological and biochemical features of the pathogen are of immense use in understanding the nature of the pathogen. Not much work has been done on these aspect but felt necessary.

Schaad (1992) reported that, most of pathovars of *Xanthomonas campestris* can be differentiated by growth and colony morphology on different media like SX, SM, BSCAA, MXP, MD-5 and Tween. It is the common fact that, any pathogenic organism exhibits considerable variability when cultured on different artificial media. However, the information on this aspect pertaining to *Xanthomonas axonopodis* pv. *punicae* is very meager. Hence, it is essential to study the behaviour of different isolates of pomegranate bacterium collected during survey on different selective/semi-selective medium. Further, molecular methods are also adopted in the present study as quick and accurate methods of detecting variability among the different isolates of the pathogen.

Now, it is the established fact that, weather factors play an important role in the inhibition and spread of the disease. In recent days, lot of emphasis is being given on weather based forecasting models for prediction of disease outbreak. Similarly need was felt to study the weather relationship of the disease, so as to predict, forecast and plan for effective protection measures.

There is ample information available on the source of survival of the pathogenic bacterium in the infected plant residues. The vital role of infected fallen leaves in the survival of pathogenic bacterium causing leaf spot of various crops is well established (Burkholder, 1948). Similarly, the infected plant residues of pomegranate, such as leaf, stem and fruit, which are left out in the field after the harvest of the crop serve as a primary source of inoculum for subsequent season to initiate the disease. But, the information on period of survival of the pathogen is still in question. Hence, the concerned aspect needs further investigation.

The continuous presence of the pathogen in the garden throughout the season has led to many fold speculations on possible survival of the pathogen on some alternate hosts grown in and around the garden. So, identification of alternate host/s is also an essential part of this investigation.

There is little information available on chemical 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.

Many a times, plant nutrients also play an important role in susceptibility or resistant mechanism of the host to different pathogens. Research on this aspect needs to be triggered to find out actual role of plant nutrients in bacterial blight development/suppression. Keeping all these aspects in view, the investigation was undertaken with the following objectives.

1. Survey and surveillance of bacterial blight of pomegranate.
2. To study the cultural, morphological, biochemical and physiological features of the pathogen.
3. To know the cultural and molecular variability of the pathogen.
4. To study the epidemiology of disease.
5. To formulate integrated strategy for the effective disease management.

2. REVIEW OF LITERATURE

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*, once deemed as a disease of minor importance, became a serious threat for pomegranate production in recent years. The disease assumed its severity in all the growing areas of Maharashtra, Karnataka and Andhra Pradesh resulting severe yield losses both in terms of quality and quantity. The information available on this disease, pathogen and management strategies are 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. Microscopic examination of lesions revealed the presence of myriads of bacteria. Later, Hingorani and Singh (1959) took the thorough investigation of the disease and pathogen, reported the presence of disease in different parts of the country.

Rangaswamy (1962) observed bacterial leaf spot of pomegranate at Annamalainagar of Madras state. The disease was reported by Sohi *et al.* (1964) in Solan region of Himachal Pradesh. Infection was generally observed on leaves but in few cases fruits were also attacked.

First record of the bacterial blight on pomegranate in Karnataka was by Chand and Kishun (1991). They have noticed 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 during 2002 (Anon., 2002) in major pomegranate areas of Bellary and Bijapur districts causing severe losses both in terms of yield and quality. Pomegranate, the boon commercial fruit crop to the farmer turned as a big bane after the outbreak of bacterial blight.

2.2 Survey and surveillance 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.*, 1991a).

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.

2.3 Symptomatology

As early as in 1944, Brichet reported *Bacterium tumefaciens* on four to five years old pomegranate plants in Algeria, which induced crown gall led to the reduction in growth and development of the plant. During 1952, Hingorani and Mehta described the symptoms as irregular spots varying from 2 mm to 5 mm in diameter, primarily appeared on the leaves. Adjacent spots coalesced and covered 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. Spots were not noticed on twigs, branches and fruits.

Rangaswamy (1962) described the symptoms of bacterial blight on leaves as necrotic spots surrounded by chlorotic halos with translucent water soaked appearance. Under severe infections, leaves became distorted and shed off. Fruits also 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. In the beginning, spots on leaves were small, circular with yellowish border and brown centre. Later on, number of adjacent spots coalesced with each other and formed elongated and irregular lesions. In many cases, spots were restricted on leaves by veins, resulted into 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 black 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 fruits coalesced together and formed irregular lesions, as a result, skin of the fruit became rough. Under heavy infection condition, fruits remained undeveloped and deformed.

In 1993, Kishun 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 appeared 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 size increased with age leading to L, Y or star shaped crackings within the spots. Severely infected fruits split opens, partially exposing the arils. Bacterial lesions were also noticed on twigs/branches resulting death of the branches. Affected plants looked unthrifty, weak and died later on.

2.4 Isolation and pathogenicity

Hingorani and Mehta (1952) isolated the pathogen from the infected 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 artificially inoculated young potted plants, 7 - 10 days after incubation. Isolation from the infected plants yielded an organism similar to one used in the inoculation experiments.

Isolation and pathogenicity studies were also carried out by Kanwar (1976). Isolation of the pathogen 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 incubation at 25 – 30^0C temperature. Pure cultures were established by repeated single colony transfer method. For pathogenicity test, inoculation was made by spraying bacterial suspension of 48 hours 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. Organism reisolated from artificially inoculated plants was found identical with original one.

Chand and Kishun (1991) standardized the inoculation method 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 cent leaf area within 21 days. The automization of bacterial suspension was found to induce lowest infection of 6 to 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 pathogen from infected leaf, twig and fruit samples of pomegranate collected from different areas of Karnataka. 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 days old plants of variety Jyothi.

2.5 Identification of the pathogen

2.5.1 Morphological characters

Hingorani and Singh (1959) described the morphological characteristics of the bacterium isolated from infected leaves of pomegranate. The bacterium was short rod with rounded ends, single or in pairs, sometimes in chains, no involution formed, measuring 1 to 2.5×0.5 µm in size, motile with a single polar flagellum, Gram negative, no endospores, capsule present, not acid fast. It readily stains with common dyes like gentian violet and carbolfuchsin.

According to Kanwar (1976), pathogen occurred in single pairs and also in chains, rod shaped with rounded ends, measured 0.75 to 3.0 µm in length and 0.45 µ in width, Gram negative with single polar flagellum, neither capsule nor endospore was observed.

Manjula (2002) reported that, seven isolates of the pomegranate bacterium were small rods, appeared singly, rarely in pairs, Gram negative, non-capsulated and no spore forming with monotrichous flagellation.

2.5.2 Biochemical characters

According to Hingorani and Singh (1959), the pathogen utilizes xylose, glucose, mannose, galactose, sucrose, lactose and raffinose but not maltose, glycerine and salicin when grown in Durham's fermentation tubes containing one per cent carbohydrates in a peptone free synthetic liquid medium. Ammonia was produced in peptone water after 15 days. Nitrites, hydrogen sulphide and indole were not produced. Starch was hydrolysed, methyl red and Voges Proskauer tests gave negative results. Growth on gelatin slabs was good. Stratiform type of liquefaction commenced after 48 hours and completed within 21 days. The yellow colour of the growth on gelatin gradually changed from usual bright yellow to dark brown on yeast glucose chalk agar and cooked potato. This discolouration was a specific character and in view of this, the pomegranate bacterium was designated as *Xanthomonas punicae* sp. *nov*. and was a new one.

Xanthomonas species readily utilize sugars in synthetic media resulting in acid formation. Production of gas during carbon utilization was absent in authentic *Xanthomonas* species. The inability to utilize salicin was a diagnostic character valuable in identifying *Xanthomonas* species (Patel *et al*., 1965).

Kishun and Chand (1991) reported that, *Xanthomonas campestris* pv. *campestris* on radish was negative in nitrate reduction, urease oxidative, fermentative metabolism of glucose and acid from adonitol and sorbitol. The bacterium was positive in H_2S production, starch hydrolysis, KOH solubility, gelatin liquefaction, hydrolysis of Tween 80, sucrose utilization, indole production, growth at 3.5 per cent NaCl, milk proteolysis and acid from most of the sugars.

All the isolates of pomegranate bacterium were positive to Xanthomonadin, tween 80 hydrolysis, gelatin liquefaction, milk proteolysis, H_2S production and catalase. It was negative in urease, arginine dehydrolase, indole, V.P. test and fermentative metabolism. Acid but no gas was produced from glucose, arabinose, mannose, cellobiose, trehalose, dextrin, glycerol, fructose, lactose, ribose, melibiose, raffinose, sucrose, salicin, mannitol, asculin and oxylose. Adonitol, sorbitol and dulcitol, were found negative for acid and gas. Bacterium was found to

utilize glucose, fructose, mannose, sorbose, ribose, trehalose, galactose, raffinose, starch and mannitol, while melonate, salacin and asculin could not be utilized (Chand and Kishun, 1991).

According to Vauterin *et al.* (1995), *Xanthomonas axonopodis* can be distinguished from other *Xanthomonas* spp. by the presence of metabolic activity on the carbon substrates *viz.,* dextrin, cellobiose, maltose, gentibiose, D-trehalose *etc.* But, lack of metabolic activity on carbon substrates such as L-rhamnose, galacturonic acid, alanine, thymine *etc.* and they designated the pathogen causing leaf spot of pomegranate as *Xanthomonas axonopodis* pv. *punicae*.

2.5.3 Cultural studies

2.5.3.1 Cultural characteristics of the pathogen

Cultural characters of the pathogenic bacterium were studied on various media by different workers. Hingorani and Singh (1959) opined that nutrient dextrose agar, yeast glucose chalk agar and potato cylinders are the best media for the cultivation of pomegranate bacterium, because of luxuriant growth obtained on them. The pathogen is facultative anaerobe. They also described that, colonies on nutrient dextrose agar were filiform, slightly raised, glistening, pale yellow and odourless. Similar characters were also found on yeast glucose chalk agar with an exception that colour of the colony was bright yellow in the beginning and gradually changed to quite dark brown with age. Growth was very poor in nutrient agar.

In 1991, Chand and Kishun reported that, they recovered pathogen from leaf, fruit and node. All the infected parts of pomegranate *viz*., leaf, fruit and node yielded mucoid, circular, convex, yellow, round, glistening and raised colonies on nutrient agar medium. On SX agar, pathogen produced a clear starch digestion zone. On the basis of pathological, cultural, biochemical and physiological characters, the authors identified the organism as *Xanthomonas campestris* pv. *punicae* (Hingorani and Singh) Dye.

Sushma Joseph (1997) recorded the highest recovery of the colonies of *Xanthomonas campestris* pv. *dieffenbachiae* of anthurium on tween media followed by cellobiose starch medium.

Shobha (1998) reported that, the recovery of bacterial colonies of *Xanthomonas axonopodis* pv. *glycines* on NSCAA, the semi-selective media were more (112×10^5 cfu/ml) as compared to nutrient agar (55×10^5 cfu/ml). But, on SX and XTS media, the bacterium produced bigger colony as compared to NSCAA medium.

2.6 Cultural variability

Venugopal *et al.* (1991) opined that, different strains of *Xanthomonas campestris* pv. *mangiferaeindicae* isolated from six varieties of mango differed culturally on different media. Strain F grew best on glucose asparagine medium, B on Dyes medium and the remaining four (ACDE) on glucose yeast extract medium.

Schaad (1992) reported that, most pathovars of *Xanthomonas campestris* can be differentiated by growth and colony morphology on semi-selective agar media like SX, SM, BSCAA, MXP, XPS, XCS, MD-5 and Tween media.

Manjula (2002) observed that, SX and BSCAA media supported the efficient growth of all the seven isolates of *X. punicae* as maximum number of colonies developed on these two media among the five semi-selective media tested.

2.7 Genetic/molecular variability

2.7.1 Random Amplified Polymorphic DNA (RAPD)

The RAPD technique has quickly gained wide spread acceptance and application because it has provided a relatively simple tool of genetic in biological system. RAPD is the best assay, when the nucleotide sequence is not known. Unlike other polymerase chain reaction (PCR) protocols, which utilize two or more primers of defined sequence, RAPD detects nucleotide polymorphism using only one primer of an arbitary nucleotide sequence.

Chakrabarty *et al.* (2004) reported that, RFLP analysis of *Xanthomonas axonopodis* pv. *malvacearum* strains from different geographical regions of the world exhibited wide degree of polymorphism and clustered them in three different groups. In addition to inter racial variability, a clear evidence of intra-racial variability was detected within race 18 isolates of *Xanthomonas*. Molecular as well as pathological evidences confirmed the existence of several biotypes within race 18 strains in cotton growing areas of India.

Siraree *et al.* (2004) generated the RAPD profiles using single decamer primers in polymerase chain reaction to study the diversity in *Xanthomonas campestris* pv. *campestris* population prevalent in the region. Amplification conditions were standardized so as to get polymorphism specific for individual isolate. Amplification products ranged from 6 to 12 with fragment size ranging from 100 bp to 5.0 kb.

2.8 Physiological characteristics

2.8.1 Temperature and pH requirement

Hingorani and Mehta (1952) found that, optimum temperature required for the growth of pomegranate bacterium was 30^0C and it tolerated upto 40^0C as maximum. Minimum temperature required for the growth is about 5 – 10^0C, the thermal death point was 52^0C.

Luxuriant growth and toxin production by *Xanthomonas axonopodis* pv. *vignicola*, causal organism of leaf blight of cowpea was recorded at a temperature of 30^0C and at pH of 7.0. Growth declined considerably at pH values higher and lower than 7.0, being minimum at pH 5.0 and 9.0 (Gour *et al*., 2000).

Manjula (2002) observed that the pathogen, *Xanthomonas axonopodis* pv. *punicae*, grew at wide temperature range of 20 to 40^0C and recorded maximum number of colonies, when inoculated plates were incubated at 27^0C. No growth of the pathogen was observed at 10^0C. Similarly, pH of 7.2 was found optimum with a range between 5.5 to 8.0.

2.9 Disease epidemiology

2.9.1 Role of weather factors

Effect of seasonal variation in temperature and humidity on bacterial leaf spot development was determined by inoculating pomegranate plants at least once a month, from March to November for three successive years. Successful infection was obtained only from middle of March to end of June, when high temperature and low humidity were normally recorded in Delhi (Hingorani and Singh, 1959).

With the results of three years experimentation on blackarm of cotton caused by *Xanthomonas malvacearum* (E. F. Smith) Downson, Lokhande and Newaskar (2000) opined that, the disease occurred during last week of August or first week of September on two months old crop, when wide spread 300 to 350 mm rainfall received, mean temperature of 22.8^0C to 31.5^0C and relative humidity between 58 to 78 per cent prevailed. Thereafter, the infection rate declined from first week of November onwards with no rainfall, decrease in mean temperature (19.1 – 30.3^0C) and relative humidity (40 – 78%).

Chand *et al.* (1991b) studied the effect of different dates of pruning on the intensity of bacterial canker of grapevine caused by *Xanthomonas campestris* pv. *viticola* and recorded the highest disease intensity (76.80%) together with maximum loss in yield in the vineyards pruned during 1 to 15th September. The disease intensity was minimum in the vineyards pruned after 10th October. The authors attributed that during early September, weather conditions were most favourable for disease development. The frequent rains during this period help pathogenesis and further spread of bacteria through splash action. The minimum (25^0C) and maximum (30^0C) temperature prevailed during first fortnight of September were also congenial for canker development. On the contrary, rains were less and eratic from October onwards.

According to Atulchandra *et al.* (1994), the disease caused by bacteria on pomegranate spreads very fast due to high temperature and low humidity from March to July months.

Misra (1995) noted that, bacterial canker of mango caused by *Xanthomonas campestris* pv. *mangiferaeindicae*, appeared with full symptom expression, every year from 1988-90 during June indicating that the weather conditions in May were congenial for disease development. The author has recorded the minimum temperature ranged between 17.0^{0}C and 19.4^{0}C, maximum temperature 40.4^{0}C and 48.0^{0}C and relative humidity between 25 to 100 per cent during May in all the consecutive years from 1988-90.

Working with bacterial spot of tomato caused by *Xanthomonas vesicatoria*, Shukla and Gupta (2005) established the highly significant and positive correlations among disease severity, soil moisture and meteorological factors such as temperature and relative humidity. They drawn the inference that, mean temperature of 28^{0}C, relative humidity of more than 90 per cent and high soil moisture with intermittent rainfall favoured the bacterial spot of tomato.

Studies conducted by Yenjerappa *et al.* (2006) revealed that the pomegranate crop pruned during first and second fortnight of September was almost free from bacterial blight incidence from pruning to harvest except that, very negligible, disease intensity on foliage was noticed in the beginning of the crop period. The reason being the uncongenial weather such as low minimum temperature (ranged between 10.8 – 19.4^{0}C) and no rainfall received (November – March) during growth and development stage of the crop.

On the contrary, the crop pruned in the month of November was absolutely free from bacterial blight infection at early stages of its growth from December to March owing to uncongenial weather prevailed, but disease started progressing from April onwards with the receipt of unusual rains and prevalence of higher temperature during April and May (maximum temperature ranged between 36.5 – 42.9^{0}C and minimum temperature between 20.8 – 24.2^{0}C). The disease severity coincided with the fruit development and fruit maturity stage, where 90 per cent of developing fruits got infected resulting the huge loss in yield and quality.

2.9.2 Survival of the pathogen

The vital role of fallen leaves in the survival of the phytopathogenic bacterium causing leaf spot disease of various crops is well established (Burkholder, 1948).

Studies conducted by Hingorani and Singh (1959) to determine the survival of pomegranate bacterium in leaves under different conditions indicated that, the pathogen survived on fallen leaves from December to mid March and reproduced from mid March to end of June.

According to Rangaswamy (1962), the pathogenic bacterium causing leaf spot of pomegranate infected through wounds and stomatal openings and causes water soaked lesions, which later develops into irregular spots. The organism spreads by air borne cells, could survive in soil for four months and cause fresh infections on new flush.

The survival of *Xanthomonas axonopodis* pv. *glycines* in soybean crop residues burried at different depths in soil was investigated by Shobha (1998). Observations revealed that, the pathogen in the infected crop residues kept on surface of soil survived beyond 20th week, survived for 16 weeks in the residue burried at 10 cm depth and could be recovered upto 10th week, when the infected residues were uniformly mixed with soil.

Upasana Rani and Verma (2002) reported that the pathogen *Xanthomonas axonopodis* pv. *punicae* survived in the infected fallen leaves kept protected under field condition upto 210 days and in canker lesions upto 80 days.

2.9.3 Host range studies

During 1959, Hingorani and Singh inoculated 59 hosts with and without injury for determining the host range of *Xanthomonas punicae*. Out of these many hosts, the pathogen attacked only *Punica granatum* L. The host plants inoculated by them were *Abelmoschus esculentus* Moench, *Amaranthus viridis* L., *Arachis hypogaea* L., *Begonia* sp., *Brassica campestris* var. *rapa* L., *Brassica oleracea* var. *botrytis* L., *Brassica oleracea* var. *capitata* L., *Cajanus cajan* L., *Capsicum frutescens etc.*

Manjula (2002) in her host range studies, inoculated all the seven isolates of pomegranate pathogen to various crops such as maize, ragi, paddy, jowar, cowpea, beans,

tomato, carrot and cabbage and found that, none of these plants were infected by any of the isolates.

Ravikumar *et al.* (2005) succeeded in establishing the host range of the pomegranate bacterium and reported the natural infection by pathogen on neem (*Azadirachta indica* L.) and bael (*Aegle marmelos* L.) grown nearby the infected pomegranate gardens. Samples were collected, analysed for symptom similarity and associated pathogen. Results on pathogenicity and cross inoculation studies confirmed the pathogen.

2.10 Integrated approach for disease management

2.10.1 Efficacy of bleaching powder and Bordeaux mixture in reducing the inoculum of the pathogen

Chlorination of drinking water has been used since long to eliminate microbial contamination. Stable bleaching powder a cheap chemical has been reported effective for the control of certain bacterial plant diseases like black rot of garden stocks (Wilson, 1942), black rot of cabbage (Patel *et al*., 1950).

Rangaswamy (1962) stated that, spraying of 5:5:50 Bordeaux mixture, one per cent perenox 1:50 lime sulphur controlled the bacterial disease on pomegranate caused by *Xanthomonas punicae*.

Thompson (1965) reported that, spread of bacterial stalk rot of corn could be checked by chlorination of water through sprinkler irrigation.

Padmanabhan *et al.* (1966) pointed out that application of bleaching powder (2 kg/ha) to the standing paddy crop, when the crop is 74 days old, controlled the bacterial leaf blight effectively and the efficacy is on par with five sprays of streptocycline + copper oxychloride.

Stable bleaching powder (250 ppm) was quite effective in reducing the number of colonies of *Xanthomonas campestris* pv. *campestris* as evaluated by Chand *et al.* (1981). The results obtained on inhibition were at par with streptocycline (100 ppm) and proved significantly superior to Agrimycin-100 (100 ppm), Agallol-3 (10,000 ppm) and plantvax 20 EC (500 ppm).

Bordeaux mixture (5:5:50) in combination with either streptocycline (500 ppm) or plantomycin (500 ppm) was very effective in reducing the citrus canker caused by *Xanthomonas citri.* Spraying of Bordeaux mixture alone also recorded the least canker incidence in comparison with bavistin @ 0.1% (Krishna and Nema, 1983).

Results on *in vitro* evaluation of chemicals carried by Shukla and Gupta (2004) indicated that, copper oxychloride (1000 ppm) in combination with mancozeb (1000 ppm) was very effective in inhibiting the growth of *Xanthomonas vesicatoria*. Bordeaux mixture (1%) was found next best effective and its efficacy was on par with streptomycin sulphate (100 ppm) + mancozeb (1000 ppm).

Jadhav and Sharma (2009) recommended the dusting of bleaching powder (copper dust, copper oxide 4%) or 20 kg/ha at least 2-3 times, in a year and spraying of Bordeaux mixture (1%) at monthly intervals during the resting period to minimize the inoculum of pomegranate bacterial pathogen.

2.10.2 Effect of stem treatment of bactericides against the infection on stem

Management of canker and gummosis diseases of citrus through stem pasting, soil drenching and aerial spraying of chemicals was tried by Jadeja *et al.* (2000). Among the different treatments, highest canker control was recorded in the plots, wherever spray application of streptomycin sulphate (100 ppm) in combination with copper oxychloride (0.2%) during June, August and December months was taken up. Bordeaux paste application and soil drenching of metalaxyl + mancozeb or fosetyl Al either alone or in combination with foliar application of streptomycin sulphate (100 ppm) + copper oxychloride (0.2%) significantly reduced gummosis incidence.

2.10.3 Evaluation of bactericides / antibiotics

2.10.3.1 *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 obtained 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.

2.10.3.2 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) investigated 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, one 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*.

2.10.4 Biological control

2.10.4.1 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.* (1991a) 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.

2.10.4.2 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, Bacillus circulans, Bacillus 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.

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

2.10.5.1 *In vitro* studies

Aqueous extracts of two plant species *viz*., *Allium sativum* (clove) and *Artabotrys uncinatus* (leaves) were tested *in vitro* for their activity against *Xanthomonas campestris* pv. *oryzae*, the causative agent of bacterial leaf blight of rice using the agar plates containing tetrazolium chloride agar. Both the extracts exhibited superior efficacy by producing largest inhibition zone against the growth of the pathogen (Grainge *et al*., 1985).

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 miswak, tulsi and patchouli, whereas miswak 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*.

2.10.5.2 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.

Ashok Kumar (2006) evaluated leaf extracts of ten different plants including obnoxious weeds for their efficacy against bacterial blight of rice. Crude extract (1%) of each ten plant species was sprayed on to the plants of susceptible rice cultivar Kasturi at the maximum tillering to booting stage of the crop. After 24 hours, sprayed plants were clip inoculated with the bacterial suspension. The author noticed that, there was a significant reduction in the disease intensity of bacterial blight in all the treatments with maximum

disease control of 58.7 per cent obtained with treatment of *Lantana camera* followed by *Eucalyptus citrodora, Eucalyptus odenophorum* and *Agave americana*.

2.10.6 Role of plant nutrition on bacterial blight development

To know the effect of nitrogen on the intensity of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in rice, Reddy *et al.* (1979) carried out the trials in both dry and wet season. Different levels of nitrogen *viz*., 60, 120 and 180 kg per ha were applied in three split doses at early tillering, tillering and panicle initiation stage and artificial inoculation with the pathogen was done at panicle initiation stage. Results obtained that, wet season was found favourable for the disease development as maximum disease severity was recorded in comparison with dry season. However, irrespective of amount of severity, disease increased significantly with increased doses of fertilizers in both the seasons.

Strong correlation between boron uptake in leaf tissues, residual boron in soil with black rot incidence in cauliflower was reported by Kumar and Kotur (1989). Exogenous supply of boron (0 – 6.4 mg/kg) in low boron containing altisols (hot water soluble soil containing boron of 0.1 mg/kg) indicated that, this micronutrient has a definite role in susceptibility of cauliflower to black rot caused by *Xanthomonas campestris* pv. *campestris*. Susceptibility was greatly observed in boron deficient (below 0.4 mg/kg) and boron excess (above 1.6 mg/kg) plants than the plants grown with optimum level of boron (0.4 – 1.6 mg/kg).

Ansari and Shridhar (2001) studied the effect of iron on the virulence of *Xanthomonas oryzae* pv. *oryzae* by pot culture experiment using susceptible (IR-8) and resistant (DV-85) rice cultivars. The pathogen was grown in medium amended with different levels of $FeSO_4$ so as to supply the iron at required concentrations *viz*., 0.20, 2.00, 4.00, 8.00 mg per lit. Twenty five days old rice seedlings were clip inoculated with 48 hours old culture grown in the medium amended with different levels of iron. The authors noticed that, increase in the concentration of iron increased the lesion length in both susceptible and resistant cultivars. Pathogen grown in iron starved medium produced minimum lesion length. Increase in lesion length was observed upto 8.0 mg per lit in susceptible cultivar but restricted upto 4 mg per lit in resistant cultivar.

2.10.7 Evaluation of integrated disease management strategy

Jadeja *et al.* (2000) achieved the successful control of citrus canker (*Xanthomonas axonopodis* pv. *citri*) with the adoption of integrated measures involving scarping of diseased portion on the main trunk, followed by pasting with Bordeaux paste and foliar application of streptomycin sulphate (100 ppm) + copper oxychloride (0.2%) three times a year.

Das and Shyam Singh (2003) recommended the integration of chemicals and cultural practices for the management of bacterial canker of acid lime. According to them, pruning of infected twigs and combined application of streptocycline (100 ppm), copper oxychloride (0.3) and neem cake (5%) for four times at monthly interval with first spray before the onset of monsoon reduced the disease significantly to the extent of 53.2 per cent over control.

From the results of five years pilot demonstration on the management of bacterial canker in acid lime, Gopal *et al.* (2004) emphasized the need of integrated approach involving cultural and chemical treatments. The technology demonstrated was pruning dried and cankerous twigs with immediate spray of copper oxychloride (0.3%) followed by two sprays of streptocycline (100 ppm) + copper oxychloride (0.3%) at monthly interval starting from June effectively reduced the canker on twig, foliage and fruit with high yield of cankerless, good marketable fruits, which benefited the farmer with cost benefit ratio of 1:1.59.

Method demonstration in farmers field comprising practices like clean sanitation, removing water shoots, spraying streptocycline, pasting of stem and branches with copper oxychloride + carbaryl + DDVP + sticker *etc.* were carried out in a campaign organized against oily spot disease of pomegranate, which benefited growers at large (Anon., 2005b).

David (2005) reported the successful management of leaf blight of onion (*Xanthomonas axonopodis* pv. *alli*) with the integration of different strategies such as use of plant activator, biological and chemical control. He opined that, integration of acibenzolar-S methyl (plant activator), biological control agents (commercial formulations of both *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A 506) with copper hydroxide in a carefully timed spray program can eliminate the leaf blight disease of onion effectively.

3. MATERIAL AND METHODS

The materials used and techniques adopted during the course of investigation are described in this chapter.

The present investigations were carried out during 2006-07 and 2007-08. Laboratory experiments were carried out at Krishi Vigyan Kendra, Hagari (Bellary district) and Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. Studies pertaining to molecular analysis were carried out at Indian Institute of Horticultural Research, Bangalore. All the field experiments were conducted in farmers' fields at Kappagal (Bellary district) and L. B. Nagar (Anantapura district) villages.

General procedure

Cleaning of glasswares

Borosil and Corning glasswares were used for all the laboratory experimental studies. They were kept for a day in cleaning solution, prepared by dissolving 60 g of potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulphuric acid (H_2SO_4) in one litre of water. Each of these chemicals dissolved separately in 500 ml of water and finally mixed. Then glasswares were cleaned by washing with detergent solution followed by tap water and finally rinsing in distilled water.

Sterilization

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.

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

Fixed plot survey was conducted for two years during 2006 and 2007 to know the incidence and severity of bacterial blight in major pomegranate growing areas comprising Bagalkot, Bellary, Bijapur, Chitradurga and Koppal districts of Karnataka and border villages of Andhra Pradesh belongs to Anantapur district. Survey was taken up in all the cropping seasons *viz.,* Mrigbahar, Hastbahar and Ambiabahar on major varieties, Bhagwa and Ganesh. The total of 172 orchards belonging to 44 villages of 11 taluks coming under 6 districts were covered during the survey. 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).

Grade	Per cent infection	
	Leaf	Fruit
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 on fruits and per cent disease index (Wheeler, 1969) on fruits and leaves was calculated by applying the formula given below.

$$\text{Per cent incidence} = \frac{\text{Number of fruits infected}}{\text{Total number of fruits}} \times 100$$

$$\text{Per cent disease index} = \frac{\text{Sum of individual disease ratings}}{\text{Total number of fruits/leaves examined}} \times \frac{100}{\text{Maximum grade}}$$

During survey, characteristic symptoms of the disease were studied, infected plant parts from all the areas of survey were collected for isolation of the pathogen and further studies.

3.2 Isolation of the pathogen

Infected plant parts such as leaf, bark of the stem and fruit pericarp showing typical symptoms of bacterial blight were subjected to isolation of causal agent. The diseased samples were washed thoroughly with tap water and allowed to dry under shade. The infected portion along with healthy part was cut into small pieces and were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for one minute and washed three times serially in sterile distilled water to remove the traces of mercuric chloride. The diseased bits were then suspended in a test tube containing 3 ml of sterilized distilled water and squeezed gently with sterilized scalpel. When the water became slightly turbid due to oozing of bacterial cells, the suspension was serially diluted upto 10^3 dilutions in 9 ml sterile water blanks. One ml of diluted bacterial cell suspension was poured into the sterilized petriplates and then 20 ml of sterilized luke warm nutrient agar medium was poured. The plates were rotated gently in clockwise and anticlock-wise direction to allow the uniform distribution of bacterial cell suspension in the medium. The inoculated plates were incubated at 30^0C for 72 hours. After the incubation period, observations were made for the development of well separated, typical, light yellow coloured bacterial colonies resembling *Xanthomonas* sp.

3.2.1 Purification and maintenance of bacterial culture

The suspected bacterial colonies were picked up with the help of sterilized inoculation loop and streaked onto the surface of Yeast extract dextrose calcium carbonate agar (Schaad and Stall, 1988) sterilized petriplates. The inoculated plates were incubated at 30^0C for 72 hours. Observations were made for the development of well separated typical, bright yellow, mucoid colonies, such pure colonies were further streaked onto the agar slants containing the nutrient agar medium and incubated at 30^0C for 72 hours, then cultures were stored in the refrigerator at 5^0C, which served as a stock culture for further studies.

The 20 bacterial cultures obtained upon isolation from the diseased samples of all the regions (surveyed) were designated as different isolates from Xa_1 to Xa_{20} and were maintained in the same way for further use.

3.2.2 Proving pathogenicity

Pomegranate plants of most susceptible variety Bhagwa to bacterial blight were raised first in polythene bags by planting apparently healthy layered cuttings. One month old saplings were then transplanted into the pots containing sterilized soil amended with necessary nutrients. The seedlings were regularly watered and exposed to sufficient sunlight.

To prove the pathogenicity of each isolate collected from different regions, the isolates were separately multiplied in nutrient broth (20 ml) taken in Erleyenmayers flask by inoculating a loopful of bacterial culture. The inoculated flasks were incubated for three days at 30^0C.

Two months old pomegranate plants were first provided with water spray and then covered with a polythene sheet for 24 hours before inoculation. The leaves of the pre-incubated pomegranate plants were slightly injured with a sterilized pin and cell suspension of the bacterial isolates adjusted to 5×10^6 cfu per ml was sprayed onto surface of the leaves with an automizer.

The sprayed plants were covered with a polythene sheets and kept in humid tent for the next 72 hours, in which humidity was maintained by constant spraying of water at 25 to 30^0C. Plants similarly sprayed with sterilized distilled water served as a control.

The plants were then taken out from the humid tent and kept openly in glasshouse. Observations were made regularly for the appearance and development of symptoms. Upon the expression of symptoms by artificially inoculated leaves, reisolation of the pathogen was done and culture so obtained was compared with the original culture.

3.3 Identification of the pathogen

The identification of the pathogen involved in causing of bacterial blight in pomegranate was determined by conducting studies on its morphological, biochemical, cultural and physiological features of the pathogen as per standard microbiological procedures.

3.3.1 Morphological characters

The morphological characteristics of the pathogen such as cell shape, gram reaction, pigmentation, capsule and spore staining characters were studied as per the standard procedures described by Anon. (1957), Bradbury (1970) and Schaad (1992).

3.3.2 Biochemical characters

The biochemical characters such as hydrolysis of starch, gelatin liquefaction, hydrogen sulphide production, catalase, oxidase and acid production from different sugars *viz.,* glucose, mannose, lactose, fructose, sucrose, mannitol, maltose, dextrose by the pathogen were studied as per the methods described by Salle (1961) and Schaad (1992).

3.3.3 Cultural studies

3.3.3.1 Cultural characters

Growth characteristics of the pathogen were studied by using various differential and selective/semi-selective media developed by the different workers. The experiment was carried out using completely randomized design (CRD) and data were analysed statistically.

The composition of the various media used in the investigation are as follows.

Modified D-5 medium

D cellobiose	: 10.00 g*
K_2HPO_4	: 3.00 g
NaH_2PO_4	: 1.0 g
NH_4Cl	: 1.0 g
$MgSO_4.7H_2O$	: 0.3 g
Cycloheximide	: 0.2 g*
Agar	: 15 g
Distilled water	: 1000 ml

*Filter sterilized (0.22 µm) D-cellobiose and cycloheximide were added after autoclaving.

XTS agar

Nutrient agar	: 23.0 g
Glucose	: 5.0 g
Distilled water	: 1000 ml
Addition after autoclaving	
Cycloheximide	: 2.0 ml*
Gentamycin	: 0.5 ml**
Cephalexin	: 1.0 ml ***

The following stock solutions were prepared.

* : 1.0 g to 10 ml of 75% ethanol

** : 50 mg to 5 ml of 75% ethanol

*** : 50 mg to 5 ml of 75% ethanol

Tween medium

Peptone	: 10.0 g
Potassium bromide	: 10.0 g
Calcium chloride	: 250.0 mg
Agar	: 15.0 g
Distilled water	: 1000 ml
Addition after autoclaving	
Tween 80	: 10.0 g
Cephalexin	: 25.0 mg
5-fluorouracil	: 6.0 mg
Tobramycin	: 0.4 mg
Cycloheximide	: 75.0 mg

BSCAA medium

Starch (soluble potato)	: 10.0 g
Glycine	: 0.2 g
K_2HPO_4	: 1.0 g
KH_2PO_4	: 1.0 g
$MgSO_4.7H_2O$	: 0.2 g*
Methyl green	: 0.2 ml**
Agar	: 15.0 g
Distilled water	: 1000 ml
Addition after autoclaving	
Cycloheximide	: 5.0 ml ***

* : Add first and be sure it is fully dissolved
** : 1% aqueous solution
*** : Add 5.0 g to 10 ml methanol, bring to 100 ml with water and filter sterilize (0.22 μm membrane)

SX agar

Starch (soluble potato)	: 10.0 g
Beef extract	: 1.0 g
Ammonium chloride	: 5.0 g
Dipotassium phosphate	: 2.0 g
Methyl violet 2B	: 1.0 ml*
Methyl green	: 2.0 ml**
Agar	: 15.0 g
Distilled water	: 1000 ml
Addition after autoclaving	
Cycloheximide	: 5.0 ml***

* : 1% solution in 20% ethanol used
** : 1% aqueous solution is used
*** : Added 5.0 g to 10.0 ml methanol, brought to 100 ml with water and filter sterilized by 0.22 μm membrane

Glucose Yeast Chalk Agar (GYCA)

Yeast extract	: 5.0 g
Glucose	: 5.0 g
Calcium carbonate	: 40.0 g
Agar	: 15.0 g
Distilled water	: 1000 ml

Before solidifying, mix well on vortex mixer and set quickly in cold water to avoid settling of $CaCO_3$.

Yeast Extract Dextrose Calcium Carbonate Agar (YDCA)

Yeast extract	: 10.0 g
Dextrose (glucose)	: 20.0 g
Calcium carbonate (USP light powder)	: 20.0 g

Agar :15.0 g
Distilled water : 1000 ml

Nutrient Sucrose Agar (NSA)

Peptone : 10.0 g
Beef extract : 5.0 g
Sucrose : 20.0 g
Agar : 15.0 g
Distilled water : 1000 ml

Yeast Extract Nutrient Agar (YNA)

Yeast extract : 5.0 g
Nutrient agar : 23.0 g
Distilled water : 1000 ml

Nutrient Agar (NA)

Beef extract : 3.0 g
Peptone : 5.0 g
Agar : 15.0 g
Distilled water : 1000 ml

The media were prepared and sterilized in an autoclave for 15 minutes at 1.1 kg per cm^2 pressure. The necessary antibiotics were filter sterilized through 0.2 µm millipore filter and added to cooled sterilized medium. The media were poured into the sterilized petriplates and dried at room temperature to eliminate the surface moisture before use.

A loopful of 72 hours old bacterial culture was added to 10 ml of sterile water blank and one ml of this suspension was further serially diluted using 9 ml sterile water blanks and 500 µl of 10^5 dilution was plated onto the surface of each of the medium contained separately in petriplates. The suspension was uniformly spread over the surface of the medium with sterilized spreader to obtain well separated bacterial colonies. The inoculated plates were incubated at 30^0C for 72 hours. Observations were drawn for number, size of colony and colony characters of the pathogen.

3.4 Cultural variability

Cultural variability among the different isolates of the pathogen was studied on five selective or semi-selective media *viz.,* modified D-5 Tween 80, XTS, SX and BSCAA.

List of isolates collected from different regions is as follows,

Isolate number	Origin/Place of collection	Taluk	District
Xa_1	S. J. Kote	Bellary	Bellary
Xa_2	Halakundi	Bellary	- do -
Xa_3	Kappagal	Bellary	- do -
Xa_4	Kurgod	Bellary	- do -
Xa_5	Uppinayakanahally	Hagaribommanahally	- do -
Xa_6	Shivanandanagar	Hagaribommanahally	- do -
Xa_7	Byasidageri	Hagaribommanahally	- do -
Xa_8	Koppal	Koppal	Koppal
Xa_9	Guttur	Koppal	- do -
Xa_{10}	Kustagi	Kustagi	- do -
Xa_{11}	Yalaburti	Yalaburga	- do -
Xa_{12}	Yalaburga	Yalaburga	Koppal
Xa_{13}	Hiriyur	Hiriyur	Chitradurga

Xa_{14}	Mollakalmuru	Mollakalmuru	- do -
Xa_{15}	Kaladagi	Bagalkot	Bagalkot
Xa_{16}	Ankalagi	Bagalkot	- do -
Xa_{17}	Jumnal	Bijapur	Bijapur
Xa_{18}	Tikota	Bijapur	- do -
Xa_{19}	L. B. Nagar	Kalyandurga (Andhra Pradesh)	Anantapur (AP)
Xa_{20}	Hirehal	Rayadurga (Andhra Pradesh)	- do -

3.5 Molecular variability

3.5.1 Molecular differentiation among the different isolates of *Xanthomonas axonopodis* pv. *punicae*

Random amplified polymorphic DNA (RAPD) analysis was used to detect the variation/s among the different isolates of *X. axonopodis* pv. *punicae*.

The RAPD technique involved the following major steps.

I) Extraction of genomic DNA (template DNA)
II) Optimization of PCR conditions
III) Gel electrophoresis
IV) Analysis of finger prints

Requirements (reagents and primers)

DNA extraction

Lysis buffer:40 mM Trisbase,20 mM sodium acetate,1 mM EDTA,1% SDS and pH 8.0
Phenol : Chloroform : isoamyl alcohol (25:24:1)
Other reagents : RNAse, 1xTE buffer, 95% ethanol, 5 M NaCl.

RAPD analysis

Template DNA random decamer primers, 10 nm dNTPs (dATP, dGTP, dTTP), Taq DNA polymerase, TAE buffer 50x, nuclease free water, Taq DNA polymerase buffer (10x). Agarose loading dye (6x), Ethidium bromide, Molecular weight markers *etc.* were used.

Instruments

Thermal cycler, centrifuge, micropipettes, gloves, pre-autoclaved micro tips, eppendorf tubes, PCR tubes, electrophoresis unit.

I. Extraction of template DNA from isolates of *X. axonopodis* pv. *punicae*

Procedure

1. Pure culture of each isolate was streaked onto the nutrient agar plates and inoculated plates were incubated at 30^0C for 72 hours.
2. Single colony of each isolate grown on NA plates was inoculated to 10 ml of nutrient broth taken in 100 ml flasks. The flasks were kept for incubation at 30^0C for 72 hours with vigorous shaking at a speed of 120 rpm.
3. About 1.5 ml aliquots of broth culture were taken in 2.0 ml eppendorf tubes and centrifuged at 13000 rpm for 5 minutes.
4. The supernatant was poured off, 200 µl of lysis buffer was added to the tubes containing pellet and was mixed well, 66 µl of 5 M NaCl was added and mixed well, contents were centrifuged at 13000 rpm for 10 minutes.
5. Supernatant (250 µl) obtained was transferred to a new tube, to which 1 µl of RNAse A (10 mg/ml) was added, mixed well and incubated at 37^0C for 30 minutes.
6. An equal volume of chloroform / isoamyl alcohol was added, mixed gently by inverting the tubes, centrifuged at 13000 rpm for 6 minutes.
7. The upper aqueous phase was transferred to a clean tube, 1.0 ml of cold 95 per cent ethanol was added, mixed well but gently. The tubes were then kept in deep freezer at -20^0C for 1 hour, centrifuged at 1300 rpm for 6 minutes.

8. Ethanol was poured off, DNA pellet was air dried by using speed vacuum for five minutes.
9. Pellet was resuspended in 50 µl of 1xTE buffer, kept in the refrigerator at 4^0C for overnight, stored in deep freezer at -20^0C.

II. Optimization of PCR conditions

The PCR amplification for RAPD analysis was performed according to Williams *et al.* (1990) with certain modifications. The optimum specifications followed for DNA amplifications were as follows.

Sl. No.	Step	Temperature (^{0}C)	Duration (min)	Number of cycles
1.	Denaturation	94	4	1
2.	Denaturation	94	1	40
3.	Annealing	37	1	
4.	Extension	72	2	
5.	Final extension	72	7	1
6.	Hold temperature	4	-	-

Primer details

A total of 20 random primers with the following sequences belonging to Operon A, B and F series were used in the study.

Sl. No.	Primer	Sequences
1.	OPA-01	5 CAG GCC CTT C-3
2.	OPA-03	5 AGT CAG CCA C-3
3.	OPA-04	5 AAT CGG GCT G-3
4.	OPA-06	5 GGT CCC TGA C-3
5.	OPA-09	5 GGG TAA CGC C-3
6.	OPA-12	5 TCG GCG ATA G-3
7.	OPA-19	5 GTT GCG ATC C-3
8.	OPA-20	5 TCG GCG ATA G-3
9.	OPB-02	5 TGA TCC CTG G-3
10.	OPB-03	5 CAT CCC CCT G-3
11.	OPB-04	5 GGA CTG GAG T-3
12.	OPB-05	5 TGC GCC CTT C-3
13.	OPB-06	5 TGC TCT GCC C-3
14.	OPF-07	5 CCG ATA TCC C-3
15.	OPF-02	5 GAG GAT CCC T-3
16.	OPF-04	5 GGT GAT CAG G-3
17.	OPF-06	5 GGG AAT TCG G-3
18.	OPF-07	5 CCG ATA TCC C-3
19.	OPF-09	5 CCA AGC TTC C-3
20.	OPF-10	5 GGA AGC TTC G-3

Master mix for PCR

Reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing the following components. The total volume of each reaction mixture was 20 µl.

1. 10x assay buffer with 15 mM $MgCl_2$	: 2.5 µl
2. dNTPs mix (2.5 mM each)	: 1.0 µl
3. Primer (5 pM/µl)	: 1.0 µl
4. Template DNA (25 ng/µl)	: 1.0 µl
5. Sterile distilled water	: 14.30 µl
6. Taq DNA polymerase (3.0 U/µl)	: 0.2 µl

Except template DNA, the master mix was distributed to PCR tubes (19 µl/tube) and later 1 µl of template DNA of each isolate was added separately to each of the master mix tube. Final volume was made upto 20 µl. PCR was carried out in thermal cycler with specifications as described.

III. Gel electrophoresis

Amplified DNA products were separated by agarose gel electrophoresis.

Requirements

1. Electrophoretic unit : Gel casting tray, gel combs, power pack, UV transilluminator
2. Agarose (1.5%)
3. Bromophenol blue
4. Ethidium bromide (loading dye)
5. 1x TAE buffer : 20 ml of 50x TAE was added to the 980 ml of distilled water and total volume was made upto 1000 ml.

Composition of 50x TAE buffer

Tris base	: 242 g
Glacial acetic acid	: 57.1 ml
EDTA (0.5 M, pH 8.0)	: 100 ml

Volume was made upto 1000 ml using distilled water.

Procedure

- Three grams of agarose was weighed and added to a conical flask containing 250 ml of 1x TAE buffer.
- The contents were melted by heating in an oven, the solution was stirred for proper mixing and complete dissolution of agarose.
- The agarose gel solution was cooled to about 40 to 45^0C and 2 – 3 drops of ethidium bromide (0.5 µg/ml) was added.
- Agarose gel was poured into the casting platform after inserting the comb in tray. While pouring, sufficient care was taken to prevent the formation of air bubbles.
- The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1x TAE), so as to cover the wells completely.
- About 2.5 µl of loading dye was added to each tube containing amplified DNA.
- The amplified products (20 µl) were carefully loaded into the sample wells. Electrophoresis was carried out at 60 volts until the tracking dye migrated to the end of the gel.
- Gel was viewed under UV transilluminator for DNA bands and then photographed for documentation.

IV. Analysis of fingerprints

Scoring of amplified fragments

The amplified profiles for all the primers were compared with each other and bands of DNA fragments were scored as ‘1’ for presence and ‘0’ for absence, generating ‘0’ and ‘1’ matrix. Per cent polymorphism was calculated by using the formula.

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Analysis of the profile of the amplified fragments

Pair-wise genetic similarities between isolates were estimated by DICE similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using sequential agglomerative hierarical nested (SAHN) cluster analysis of NTSYS-PC version 2.0 (Rohlf, 1998).

3.6 Physiological characters

3.6.1 Temperature requirement

The study was conducted to know the optimum temperature requirement for the growth of *X. axonopodis* pv. *punicae* using modified D-5 as basal medium.

A loopful of 72 hours old bacterial culture was serially diluted using 9 ml sterile water blanks. One ml of 10^5 dilution was plated onto the surface of nutrient agar the medium contained in sterilized petriplates and the suspension was uniformly spread over the medium with the help of sterilized spreader. Then inoculated plates were incubated at different temperature levels *viz.,* 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50^0C, respectively for 72 hours. Observations were drawn for the development of colonies in the inoculated plates kept at specific temperature levels. Colonies were counted, recorded and data were analysed as per the statistical procedures.

3.6.2 pH requirement

The effect of hydrogen ion concentration on the growth of *X. axonopodis* pv. *punicae* was studied by adjusting the pH of the medium (modified D-5) to various levels *viz.,* 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0 using appropriate phosphate buffer.

A loopful of 72 hours old bacterial culture was serially diluted to 9 ml sterile water blanks. One ml of 10^5 dilution was plated separately on to the surface of the medium having varied pH levels. The suspension was spread uniformly over the medium with the help of sterilized spreader. Inoculated plates were incubated at 30^0C temperature for 72 hours. After the incubation period, observations were recorded for the development of colonies in the media having different pH. Colonies were counted and recorded. Data were analysed statistically.

3.7 Disease epidemiology

3.7.1 Role of weather factors on bacterial blight development in pomegranate

Pomegranate is a perennial fruit crop and is grown in all the three seasons such as Mrigbahar, Hastbahar and Ambiabahar. As per the earlier literature, it is known that bacterial infection in pomegranate gets aggravated with the prevalence of high temperature, humidity, coupled with rainfall (Kishun, 1993). The present investigation was aimed at studying the effect of these environmental factors in relation to the disease.

Field trials were taken up in four cropping seasons in farmers fields at Bellary. Most susceptible variety Bhagwa was selected for the study. Crop pruned during different months *viz.,* November 2006 (late Hastbahar), April 2007 (Ambiabahar), July 2007 (Mrigbahar) and September 2007 (Hastbahar) was constantly examined for disease progress/decline. Thirty plants at random in each plots were selected, observations on the incidence and severity of bacterial blight in each plot were recorded at weekly interval right from pruning upto the harvest of the crop. Correspondingly, prevailing weather parameters during standard meteorological weeks such as maximum and minimum temperature, rainfall and relative humidity were also recorded. Data were analysed statistically and correlated with weather

factors. Original values were converted into $\sqrt{x+1}$ transformed values and further analyzed to get the estimated values for comparison.

3.7.2 Studies on survivability of the pathogen

Crop residues such as infected leaf, fruit and stem material serves as a means for survival of plant pathogenic bacteria and through which, it carryovers from season to season. The main aim of this investigation is to study the length of survival of *X. axonopodis* pv. *punicae* in the infected leaf and fruit residues of pomegranate.

The polythene bags measuring 30 × 15 cm were filled separately with sterilized and unsterilized soil collected from fields of pomegranate. Pomegranate leaf and fruit residues (fruit pericarp) showing typical symptoms of bacterial blight caused by *X. axonopodis* pv. *punicae* were collected from infected fields. The diseased leaf and fruit pericarp were chopped into small pieces and buried separately at different depths *viz.,* 5, 10, 15 cm and on top of sterilized and unsterilized soil filled in polythene bags.

All the filled in bags were kept at open conditions. Soil moisture in the polythene bags was maintained by careful watering. At regular interval of 15 days, recovery of the bacterium through isolation was carried out by taking one gram of soil sample from each polythene bag, where infected plant material was buried following the dilution plating technique on modified D-5 medium. Bacterial colonies obtained at each isolation were counted and recorded.

3.7.3 Host range of *Xanthomonas axonopodis* pv. *punicae*

The ability of the pathogen to infect the different hosts was studied by inoculating the culture of the pathogen to suspected host plants under artificial conditions. Most common weeds and perennial plants seen in and around the pomegranate fields were selected for the study.

The following plants were inoculated artificially for determining the host range.

Sl. No.	Common name	Scientific name	Family
1.	Parthenium	*Parthenium hysterophorus* L.	Asteraceae
2.	Neem	*Azadirachta indica* A. Juss	Meliaceae
3.	Sida	*Sida cordifolia* L.	Malvaceae
4.	Tridax	*Tridax procumbens* L.	Asteraceae
5.	Achyaranthes	*Achyranthes aspera* L.	Amaranthaceae
6.	Croton	*Croton sparsiflorus* Morang	Euphorbiaceae
7.	Bael	*Aegle marmelos* L.	Rutaceae
8.	Physalis	*Physalis minima* L.	Solanaceae
9.	Tinospora	*Tinospora cordifolia* (Willd) Miers	Menispermaceae
10.	Euphorbia	*Euphorbia pulcherima* Willd exklotzsch	Euphorbiaceae

The plants of these hosts were raised in pots containing sterilized soil supplied with necessary nutrients. The pathogenic bacterium was multiplied by inoculating the loopful of culture in 20 ml of nutrient broth taken in Erleyenmayer flasks. The inoculated flasks were incubated at 30^0C for 72 hours.

The plants raised in pots were provided with water spray and covered with polythene bags for 24 hours (pre-incubation) before inoculation. The leaves were slightly injured with sterilized insect pin.

The bacterial suspension (after incubation) was adjusted to 5×10^6 cfu per ml of water in spectrophotometer at 480 nm was sprayed onto the surface of injured leaves of different plants with low speed automizer. The plants after inoculation were covered with a polythene sheet and kept in humid tent for the next 72 hours, where constant humidity was maintained with frequent water spray. Plants similarly sprayed with sterilized distilled water served as a control.

After 72 hours, plants were shifted to the glasshouse. Observations were made regularly for the development of symptoms. Upon the expression of symptoms by artificially inoculated plants, reisolation and reinoculation of pathogen to the pomegranate plants was carried out for confirmation.

3.8 Integrated disease management

3.8.1 Efficacy of bleaching powder and Bordeaux mixture in reducing the initial inoculum of the pathogen

Bleaching powder (Saini and Prashar, 1981) and Bordeaux mixture (Jarial and Shyam, 2002) are the antibacterial chemicals used to control the plant pathogenic bacteria. The present investigation was taken up to assess the efficacy of these two chemicals in reducing the initial inoculum of *X. axonopodis* pv. *punicae*.

The following treatments were set up using Completely Randomized Design with four replications and there were three plants in each replication.

Tr. No.	Treatments
1	: Bleaching powder (100 g/plant)
2	: Bordeaux mixture (1%)
3	: Bordeaux mixture (1%) + bleaching powder (100 g/plant)
4	: Copper oxychloride (0.2%)
5	: Copper oxychloride (0.2%) + bleaching powder (100 g/plant)
6	: Untreated control

During rest period of the crop, after mrigbahar cropping season Bordeaux mixture and copper oxychloride were sprayed separately onto the plants having old infection on the leaf, which was not in progress. Three sprays were taken at an interval of 10 days. Soil application of bleaching powder @ 100 g per plant was taken up treatment-wise around the basin of the plants. The treated foliage (leaf) of all the treatments after defoliation was subjected for recovery of the pathogen through isolation. The colonies appeared on the medium (NA) were counted and recorded. Data obtained was statistically analysed.

3.8.2 Effect of stem treatment of bactericides on the spread of infection of bacterial blight

Infected stem/branches act as a primary source of inoculum, through which bacterial infection spreads from season to season. Eradication of stem infection by mechanical means is not possible because, stem is an integral part of the plant and it can not be removed. So pasting of bactericides/antibacterial chemicals is an alternate option.

An experiment was planned to assess the efficacy of different bactericides to prevent the spread of infection through the way of pasting these bactericides onto the stem. Field study was conducted during ambiabahar cropping season in Randomized Completely Block design with three replications having four plants for each treatment. The variety Bhagwa was selected for the study and the plants were 6 years old.

Sl. No.	Treatments
1.	Bactinash-200 (0.05%) + Copper oxychloride (0.2%)
2.	Bactrinashak (0.05%) + Copper oxychloride (0.2%)
3.	Black out (0.05%) + Copper oxychloride (0.2%)
4.	Bronip (0.05%) + Copper oxychloride (0.2%)
5.	Streptocycline (0.05%) + Copper oxychloride (0.2%)
6.	Bordeaux mixture (1%)
7.	Copper oxychloride (0.2%)
8.	Untreated control

The plot was kept clean without any other source of infection (infected leaf, fruits and other weeds were completely removed). Solution at required concentration was prepared by dissolving appropriate quantity of bactericides in water. Red oxide (20 g/lit) was added to make the solution into paste. Plants after pruning and complete defoliation (by ethryl spray of 0.3%) were pasted with different bactericides as treatment-wise. Observations were drawn for the incidence and severity of bacterial blight, one, two and three months after pasting. Yield obtained in different treatments was also recorded and data were analysed statistically.

3.8.3 Evaluation of bactericides / antibiotics

3.8.3.1 *In vitro* evaluation of bactericides / antibacterial chemicals

A set comprising six commercially available bactericides and three antibacterial chemicals each at two concentrations were evaluated for their efficacy against the growth of *X. axonopodis* pv. *punicae* by inhibition zone assay method.

The list of bactericides is as under.

Sl. No.	Bactericides/Antibacterial chemicals	Concentration (%)	
1.	Bacterinashak	0.05	0.1
2.	Bactinash 200	0.05	0.1
3.	Bronip	0.05	0.1
4.	K-cycline	0.05	0.1
5.	Plantomycin	0.5	1
6.	Streptocycline	0.05	0.1
7.	Bleaching powder	0.5	1
8.	Bordeaux mixture	0.5	1
9.	Copper oxychloride	0.2	0.3

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.

3.8.3.2 Field evaluation of bactericides and antibacterial chemicals against bacterial blight

. A set of six commercially available bactericides and three antibacterial chemicals were screened for their efficacy to check the incidence and severity of bacterial blight in pomegranate. This experiment was conducted in two seasons *viz.,* Mrigbahar (June – December) of 2007 and Ambiabahar (February – August) of 2008 in farmer fields at Kappagal (Bellary taluk) and L. B. Nagar (Kannekal taluk) villages, respectively. The trials were laid out in Randomized Completely Block Design (RCBD) with three replications and there were four plants in each treatment. The details of treatments were furnished below.

T_1 : Bactinash 200 (0.05%) + Copper oxychloride (0.2%)
T_2 : Bacterinashak (0.05%) + Copper oxychloride (0.2%)
T_3 : Bronip (0.05%) + Copper oxychloride (0.2%)
T_4 : K cycline (0.05%) + Copper oxychloride (0.2%)

T_5 : Plantomycin (0.5%) + Copper oxychloride (0.2%)
T_6 : Streptocycline (0.05%) + Copper oxychloride (0.2%)
T_7 : Bleaching powder (1%)
T_8 : Bordeaux mixture (1%)
T_9 : Copper oxychloride (0.2%)
T_{10} : Untreated control

A total of five sprays were given at an interval of 10 days with first spray at the disease onset. All the bactericides (antibiotics) were applied in combination with copper oxychloride. Spraying was done using manually operated high volume (knapsack) sprayer. Observations pertaining to the incidence and severity on fruits were recorded before and after third and fifth spray using 0 – 6 scale. Per cent disease incidence and per cent disease index was worked out. The data obtained were analysed statistically.

The data on fruit yield obtained in treated plots was also recorded and analysed as per the statistical procedures (Sukhatme and Amble, 1985).

3.8.4 Evaluation of bioagents

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

Five biocontrol agents *viz., Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescence*, *Pseudomonas putida* and *Bacillus subtilis* were evaluated for their efficacy against the growth of *X. a.* pv. *punicae* by inhibition zone assay method. The cultures / formulations of these biocontrol agents were obtained from University of Agricultural Sciences, Dharwad and T-Stanes Company Ltd., Coimbatore.

A heavy suspension (3 day old) of *X. a.* 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 30^0C for 72 hours.

Observations were recorded for the zone of inhibition produced by antagonistic microorganisms around the growth of the pathogen.

3.8.4.2 *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 Hastbahar (September – March) cropping season of 2007-08 in farmers' field at Kappagal village (Bellary taluk). The trial was laid out in randomized completely block design with four replications and there were three plants for each treatment. The following bioagents each at 0.5 per cent concentrations were screened in the study.

T_1 : *Bacillus subtilis*
T_2 : *Pseudomonas fluorescens*
T_3 : *Pseudomonas putida*
T_4 : *Trichoderma harzianum*
T_5 : *Trichoderma viride*
T_6 : Microbial consortia
(*Pseudomonas fluorescens* + *Bacillus subtilis* 1:1)

The bioagents were sprayed five times at an interval of 10 days between each spray with 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. The data obtained were analysed statistically. The data on fruit yield obtained in treated plots were also recorded and analysed statistically.

3.8.5 Effect of botanicals

3.8.5.1 *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.	Common name	Botanical name	Family	Plant parts used
1.	Onion	*Allium cepa* L.	Liliaceae	Bulb
2.	Garlic	*Allium sativum* L.	Liliaceae	Bulb
3.	Neem	*Azadirachta indica* Juss.	Meliaceae	Seed
4.	Kashmir bouquet	*Clerodendron inarmae* L.	Verbenaceae	Leaf
5.	Turmeric	*Curcuma longa* L.	Zingiberaceae	Rhizome
6.	Lantana	*Lantana camera* L.	Verbanaceae	Leaf
7.	Tulsi	*Ocimum sanctum* L.	Lamiaceae	Leaf
8.	Congress weed	*Parthenium hysterophorus* L.	Asteraceae	Leaf
9.	Zinger	*Zingibar officinale* Rosc.	Zingiberaceae	Rhizome

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. a.* 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.

Five and ten per cent each of plant extract was prepared by mixing 5 and 10 ml of stock solution with 95 and 90 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 millimeter in diameter and data obtained was analysed statistically.

3.8.5.2 Field evaluation of plant extracts

Nine plant extracts, which were evaluated *in vitro* were further subjected for field evaluation against bacterial blight of pomegranate. The study was taken up in farmer's field during Ambiabahar (February – August) cropping season of 2008. The variety Bhagwa was selected for the study and the plants were 6 years old. The experiment was laid out in Randomized Complete Block Design with three replications having four plants for each treatment.

The extracts were prepared as per the method described in previous study. All the plant extracts each at 10 per cent concentration were sprayed as soon as the initial disease symptoms were noticed on fruits. A total of five sprays were taken at an interval of 10 days. Observations on disease incidence and severity were recorded before and after the application of plant extracts using 0 to 6 scale. Fruit yield in each treatment was recorded and results were analysed statistically.

3.8.6 Effect of micronutrients on the incidence and severity of bacterial blight in pomegranate

The field study was conducted to view the significance of application of micronutrients such as zinc, boron, calcium, iron, magnesium and micronutrient combination in reducing the incidence and severity of bacterial blight in pomegranate.

The study was taken up in farmers field during Ambiabahar cropping season of 2008. The experiment was laid out in RCBD with three replications. There were four plants for each treatment. The details of treatments are furnished below.

Sl. No.	Micronutrients	Source
1.	Boron	Solu B.
2.	Calcium	Calcium sulphate ($CaSO_4$)
3.	Iron	Ferrous sulphate ($FeSO_4$)
4.	Magnesium	Magnesium sulphate ($MgSO_4$)
5.	Multinutrients (Zn, Mg, B, Fe, Cu, Mn, Mo and S)	Mazik
6.	Zinc	Zinc sulphate ($ZnSO_4$)
7.	Untreated control	-

Dosage : Soil application : (100 g/plant) Foliar spray @ 1%

Plot was kept clean and weeds free. Soil application of micronutrient was taken up one month after pruning. Micronutrients (treatment-wise) were applied to the soil around the basin of the plant. Foliar applications of the same nutrients were given four times at fruiting stage (first spray at the initial fruit set) with an interval of 10 days between each spray.

Observations on the incidence and severity of bacterial blight on fruits were drawn after second and fourth spray. Yield obtained in each treatment was recorded and results were analysed statistically.

3.8.7 Evaluation of IDM strategy

Utilizing the results obtained on various aspects of bacterial blight management in pomegranate, integrated disease management module was formulated and evaluated for its efficacy in farmers field. The treatment details of the integrated trial is narrated below.

T_1 : Integrated disease management module

a) Orchard maintenance : At the time of pruning, all the leftover fruits of previous cropping season were collected and burnt, infected stem/branches were cut and removed (wherever feasible), leaf inoculum was destroyed through the collection and burning of all the defoliated leaves after the spray of ethryl spray (3 ml/lit).
b) Soil application of bleaching powder was taken up around the basin of the plants @ 100 g/plant.
c) Stem treatment : Plants after pruning and complete defoliation were pasted with bronip (0.05%) + copper oxychloride (0.2%) and red oxide @ 20 g per lit was added to make the solution into the paste.
d) Biological control : Three sprays of *Pseudomonas fluorescens* (0.5%) were alternated with two sprays of garlic extract (10%) at an interval of 10 days with first spray at one month after pruning before the disease incidence.
e) Chemical protection : Alternate sprays with bactinash-200 + copper oxychloride (0.2%) and bronip (0.05%) + copper oxychloride (0.2%) were given. A total of five applications were taken at an interval of 10 days with first spray as soon as the disease symptoms were noticed.
f) Micronutrient application : Mazik (1%) as multinutrient was sprayed for five times at an interval of 10 days between each spray with first spray at the initial fruit set (three months after pruning).

T_2 : Farmers practice

Farmers have adapted the following technology for the control of bacterial blight of pomegranate in below mentioned seasons.

(i) Mrigbahar cropping season

1. Sprays of bleaching powder (1%) and copper oxychloride (0.2%) were alternated at 10 days interval at one month after pruning.

2. Combination of streptocycline (0.1%) + copper oxychloride (0.3%) was the next spray (at one and half months after pruning but before the disease incidence).
3. Copper oxychloride (0.3%) was sprayed 10 days after the disease incidence followed by a spray of *Pseudomonas fluorescence* (0.5%) + *Trichoderma viride* (0.5%) at an week interval.
4. Spray of bactrinashak (0.06%) was taken up (at one month after the disease incidence) followed by the spray of bleaching powder (1%) at 5 days interval.
5. K cycline (0.1%) + copper oxychloride (0.3%) was taken up at 1½ month after the disease incidence.
6. Micronutrients were applied through spray and drip, a total of 10 applications of micronutrients (calcium (2%), boron (1%), zinc (1%), iron (2%) and multinutrients (1%)) were taken up.

(ii) Hastbahar cropping season

1. Foliar application of Bordeaux mixture (1%) was taken up, before the disease incidence (one month after pruning) followed by the spray of bactrinashak (0.1%) + COC (0.3%) at 15 days interval.
2. Copper oxychloride (0.3%) was sprayed two months after pruning, when symptoms of the disease were noticed on leaves.
3. Combined application of streptocycline (0.1%) and copper oxychloride (0.3%) was taken up, when the infection is noticed on fruits (3 months after pruning), Bronip (0.05%) + COC (0.3%) was the next spray at week interval.
4. Bactrinashak (0.1%) + COC (0.3%) was sprayed, 10 days after the previous spray.
5. Micronutrients *viz.,* zinc (1%), iron (1%), calcium (2%), magnesium (2%) and multinutrients (1%) were applied for eight times (five sprays and three applications through drip) after the fruit set with one week interval between each application.

Trial was conducted in two seasons Mrigbahar and Hastbahar of 2008 on varieties Bhagwa (Mrigbahar) and Ganesha (Hastbahar). Total area of each treatment was 0.2 ha. All other practices such as irrigation, fertigation, insect pest and fungal disease control strategies were similar for both the treatments as per the recommendations.

Observations were drawn for the disease incidence and severity. Yield obtained was recorded. Economics of both the treatments were worked out.

4. EXPERIMENTAL RESULTS

The results of the investigations undertaken on "Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*" during 2006-07 and 2007-08 are presented as under.

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

Fixed plot survey was undertaken for two years during 2006 and 2007 to assess the incidence and severity of bacterial blight of pomegranate in major pomegranate growing areas of Karnataka and border villages of Andhra Pradesh. Results showed that, in general, disease incidence on fruit was more than its severity in all the areas surveyed for both the years with an exception that, severity on fruits was more than incidence in Kannal and Kannur villages of Bijapur, Govanakoppa and Udagatti villages of Bagalkot district during 2006.

During 2006 (Table 1), per cent disease index on leaf ranged from 0.00 to 42.35 with highest per cent disease index (PDI) recorded on Bhagwa variety during mrigbahar in Kannur village of Bijapur district followed by 40.36 in Shakapur (Kustagi), 37.77 in Byasidageri (H. B. Hally) and 36.74 in Kannal (Bijapur) village. No incidence on Bhagwa variety was observed in Irkalgada and Guttur villages of Koppal district.

Stem infection (bacterial lesions on the stem/branches) was also recorded. Maximum stem infection of 4.79 lesions per branch was recorded in Yadonni village of Yalburga taluk followed by 4.54, 4.32, 3.89, 3.65, 3.62 lesions per branch in Hiremonnapur (Kustagi), Hirehal (Rayadurga), Morigere (H. B. Hally), Kannur (Bijapur) and Moudigere (Kustagi) villages, respectively. Minimum lesion of <1 per branch was recorded in Irkalgad and Guttur (Koppal), Kappagal (Bellary), Govanakoppa and Udagatti (Bagalkot), Rangapur camp (Anantapur) and Jumnal and Tikota (Bijapur) villages.

Highest fruit incidence of 78.94 per cent was noticed on Bhagwa variety in Maskal (Hiriyur), followed by 74.36 in Hiremonnapur and 71.55 in L. B. Nagar (Anantapur district). It was more than 50.00 per cent in Sanjeevarayanakote, Chilgod (H. B. Hally), Kurgod (Bellary), Kannur (Bijapur), Shakapur, Kundalagere (Hiriyur), Lokapur (Bagalkot) villages. No disease was recorded in plots at Irkalgda, Guttur (Koppal) and Kappagal villages of Bellary taluk. With regard to the disease severity on fruits, highest severity of 83.75 PDI was observed in Kannur village followed by Maskal (56.76 PDI), Hiremonnapur (52.82 PDI) and Horti (51.75 PDI) with least fruit severity of 3.86 PDI recorded in Halakundi village of Bellary district and crop was found totally free from bacterial blight infection in Irkalgod and Guttur villages of Koppal district.

Looking into the district average during 2006, maximum fruit severity of 40.39 PDI was observed in Bijapur district followed by Chitradurga, Bagalkot and Anantapur districts, in which per cent disease index ranged between 24.13 to 29.47. Highest (41.41%) and lowest (29.45%) incidence on fruit was recorded in Chitradurga and Bijapur districts, respectively and it was moderate in all other districts (30% – 40%).

Similarly, survey report of 2007 revealed that (Table 2), highest leaf severity of 56.78 PDI was recorded on Bhagwa variety in Shakapur village during Mrigbahar. Disease was moderate with 31.24 to 36.27 PDI on leaf in Makalli (Yalburga), Somalapur (Rayadurga), Goanakoppa and Hiremonapur villages.

More number of lesions on the stem per branch were found in the plots located at Somalapur (3.45), Hiremonnapur (3.53) and Shakapur (4.21) villages with highest number of lesions to the tune 4.8 per branch was counted and recorded in Byasidageri village. Minimum stem infection as indicated by <1 lesion per branch was noticed in S. J. Kote, Emmiganur (Bellary), Chilgod, Guttur, Irakalgada, Maskal, Hosayalanadu (Chitradurga) and Kondlahally (Mollakalmuru) villages.

Survey report of Shivanandanagar (H. B. Hally) revealed the highest disease incidence on fruit (in comparison with all the districts) to an extent of 63.74 per cent on Bhagwa variety followed by 56.72, 56.47 and 51.23 per cent in Yalbhurti, Yadonni and Halakundi villages, respectively.

Table 1: Survey on the incidence of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in major areas of Karnataka and border areas of Andhra Pradesh during 2006

Sl. No.	District	Taluk	Village	Number of orchards	Cropping season	Variety	Leaf	Stem	Fruit	
							Per cent disease index	No. of lesions/ branch	Per cent incidence	Per cent disease index
1.	Bagalkot	Bagalkot	Ankalagi	4	Hastbahar	Bhagwa	21.35	1.27	18.62	11.29
			Govanakoppa	3	Mrigbahar	Bhagwa	18.69	0.63	11.26	14.37
			Kaladagi	8	Mrigbahar	Bhagwa	28.72	2.08	49.13	40.88
			Lokapur	3	Mrigbahar	Bhagwa	24.36	3.04	68.17	36.35
			Udagatti	2	Mrigbahar	Ganesha	33.74	0.99	21.76	25.52
2.	Bellary	Bellary	Emmiganur	3	Hastbahar	Ganesha	8.64	2.03	26.02	16.98
			Halakundi	5	Ambiabahar	Bhagwa	7.40	2.13	18.05	3.86
			Kappagal	2	Hastbahar	Bhagwa	2.22	1.26	25.62	13.34
				2	Hastbahar	Ganesha	4.53	0.00	0.00	0.00
			Kurgod	6	Mrigbahar	Bhagwa	25.67	3.82	53.08	27.18
			Sanjeevarayankote	3	Hastbahar	Ganesha	27.03	1.60	44.51	23.09
				2	Hastbahar	Bhagwa	33.57	1.20	51.72	27.59
			Vaddatti Camp	2	Ambiabahar	Bhagwa	17.78	3.57	43.38	17.18
		Hagaribommanahally	Byasidageri	3	Mrigbahar	Bhagwa	37.77	2.23	22.76	15.34
			Chilgod	4	Mrigbahar	Bhagwa	21.31	1.99	52.02	29.18
			Morigere	3	Hastbahar	Bhagwa	24.68	3.89	18.28	9.37
			Shivanandanagar	2	Hastbahar	Mrudula	11.08	2.19	-	-
				2	Hastbahar	Bhagwa	26.23	3.23	-	-
			Uppinayakanahally	6	Hastbahar	Bhagwa	18.06	2.66	68.34	40.27
3.	Bijapur	Bijapur	Horti	2	Mrigbahar	Bhagwa	26.27	2.74	29.34	51.75
			Jumnal	4	Mrigbahar	Bhagwa	31.45	0.78	31.37	18.72
			Kannal	3	Mrigbahar	Bhagwa	36.74	2.88	19.72	23.14
			Kannur	4	Mrigbahar	Bhagwa	42.35	3.65	55.26	83.75
			Tikota	3	Hastbahar	Ganesha	22.63	0.92	11.58	24.61

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Sl. No.	District	Taluk	Village	Number of orchards	Cropping season	Variety	Leaf	Stem	Fruit	
							Per cent disease index	No. of lesions/ branch	Per cent incidence	Per cent disease index
4.	Chitradurga	Hiriyur	Echalageri	4	Ambiabahar	Bhagwa	22.45	2.97	32.89	28.54
			Hosayalanadu	4	Ambiabahar	Bhagwa	29.43	1.20	48.00	24.88
			Kundalagere	3	Ambiabahar	Bhagwa	25.36	2.13	62.37	45.77
			Maskal	5	Ambiabahar	Bhagwa	34.99	1.75	78.94	56.76
		Molakalmuru	Hanagal	4	Hastbahar	Bhagwa	30.26	1.94	20.63	18.74
			Kondlahally	4	Hastbahar	Ganesha	19.29	1.52	36.29	23.54
			Rampura	2	Mrigbahar	Bhagwa	10.74	1.08	31.72	25.69
				3	Hastbahar	Bhagwa	18.34	2.51	20.43	11.81
5.	Koppal	Koppal	Guttur	2	Hastbahar	Bhagwa	0.00	0.00	0.00	0.00
			Irkalgada	4	Mrigbahar	Bhagwa	0.00	0.00	0.00	0.00
			Koppal	5	Mrigbahar	Bhagwa	7.18	2.83	13.26	7.82
		Kustagi	Hiremannapur	5	Mrigbahar	Bhagwa	20.04	4.54	74.36	52.82
			Moudigere	3	Ambiabahar	Bhagwa	16.66	3.62	44.97	32.48
			Shakapura	5	Mrigbahar	Bhagwa	40.36	1.96	57.69	38.03
			Yalabhurthi	5	Mrigbahar	Bhagwa	21.29	1.89	28.42	10.49
		Yalburga	Hirehalli	4	Mrigbahar	Bhagwa	22.59	2.85	49.56	36.87
			Makalli	3	Mrigbahar	Bhagwa	18.27	1.09	24.74	8.64
			Yadonni	4	Mrigbahar	Bhagwa	15.34	4.79	33.24	17.19
6.	Anantapur	Kanekal	L. B. Nagar	2	Mrigbahar	Ganesha	16.91	1.16	33.46	16.48
				2	Mrigbahar	Mrudula	27.34	2.53	71.55	46.92
			Rangapur Camp	4	Mrigbahar	Bhagwa	20.39	0.64	28.77	22.36
			Uddahal	2	Hastbahar	Ganesha	10.26	1.21	-	-
		Rayadurga	Hirehal	3	Mrigbahar	Ganesha	30.76	4.32	48.69	25.72
				2	Mrigbahar	Bhagwa	28.39	2.72	35.77	31.84
			Hiridal	4	Hastbahar	Bhagwa	9.62	2.07	19.50	6.08
			Somalapur	4	Hastbahar	Bhagwa	17.77	3.26	22.98	14.98

Note : Mrigbahar : Pruning from May-July
Hastbahar : Pruning from September-November
Ambiabahar : Pruning from February-April

Table 2: Survey on the incidence of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in major areas of Karnataka and border areas of Andhra Pradesh during 2007

Sl. No.	District	Taluk	Village	Number of orchards	Cropping season	Variety	Leaf	Stem	Fruit	
							Per cent disease index	No. of lesions/ branch	Per cent incidence	Per cent disease index
1.	Bagalkot	Bagalkot	Ankalagi	5	Mrigbahar	Bhagwa	23.84	0.67	36.29	8.76
			Govanakoppa	5	Mrigbahar	Bhagwa	32.68	2.83	34.89	26.45
			Kaladagi	5	Mrigbahar	Bhagwa	20.29	2.28	15.83	21.64
			Lokapur	4	Mrigbahar	Bhagwa	21.82	0.64	28.36	15.69
			Udagatti	3	Ambiabahar	Ganesha	15.06	1.29	11.54	10.27
2.	Bellary	Bellary	Emmiganur	3	Hastbahar	Ganesha	9.62	0.50	17.80	7.00
			Halakundi	4	Hastbahar	Bhagwa	8.80	2.90	51.23	18.91
			Kappagal	2	Hastbahar	Bhagwa	18.94	3.2	18.78	16.79
				2	Hastbahar	Ganesha	11.26	1.04	15.62	13.72
			Kurgod	4	Hastbahar	Bhagwa	9.25	1.86	17.19	5.10
			Sanjeevarayana Kote	2	Hastbahar	Ganesha	7.70	0.60	27.64	9.39
				1	Mrigbahar	Bhagwa	29.25	2.9	30.53	10.00
			Vaddathi Camp	2	Hastbahar	Bhagwa	2.2	3.13	2.24	0.62
		Hagaribomma nahalli	Byasidageri	3	Mrigbahar	Bhagwa	15.96	4.80	28.84	16.66
			Chilgod	2	Hastbahar	Bhagwa	13.33	0.96	19.11	11.27
			Morigere	4	Hastbahar	Bhagwa	18.94	2.65	40.29	26.35
			Shivanandanagar	4	Mrigbahar	Bhagwa	17.40	2.40	63.74	50.84
			Uppinayakanahally	4	Hastbahar	Bhagwa	1.85	1.93	8.20	2.58

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Sl. No.	District	Taluk	Village	Number of orchards	Cropping season	Variety	Leaf	Stem	Fruit	
							Per cent disease index	No. of lesions/ branch	Per cent incidence	Per cent disease index
3.	Bijapur	Bijapur	Horti	2	Mrigbahar	Bhagwa	10.74	0.86	13.25	8.72
			Jumnal	4	Mrigbahar	Bhagwa	15.54	2.16	21.26	12.65
			Kannal	3	Ambiabahar	Bhagwa	19.78	2.36	26.49	20.63
			Kannur	4	Mrigbahar	Bhagwa	28.36	2.54	46.49	31.76
			Tikota	3	Ambiabahar	Bhagwa	21.74	1.56	18.39	11.59
				1	Mrigbahar	Ganesha	28.39	2.13	35.74	19.65
4.	Chitradurga	Hiriyur	Echalageri	4	Ambiabahar	Bhagwa	7.40	1.76	51.11	35.63
			Hosayalanadu	5	Ambiabahar	Bhagwa	3.70	0.63	14.08	4.38
			Kundalagere	2	Ambiabahar	Bhagwa	8.88	2.16	17.16	11.11
			Maskal	5	Ambiabahar	Bhagwa	28.88	0.56	26.95	23.52
		Molakalmuru	Hanagal	4	Hastbahar	Bhagwa	28.37	2.26	31.26	22.74
			Kondlahally	2	Mrigbahar	Ganesha	11.26	0.49	20.35	8.59
				3	Mrigbahar	Bhagwa	32.57	2.46	56.54	38.93
			Rampura	4	Mrigbahar/	Bhagwa	26.36	2.82	63.54	51.63
				2	Hastbahar	Bhagwa	15.38	1.73	43.29	36.51
5.	Koppal	Koppal	Guttur	3	Mrigbahar	Bhagwa	8.28	0.99	15.76	8.74
			Irkalgada	4	Mrigbahar	Bhagwa	0.00	0.00	0.00	0.00
			Koppal	4	Mrigbahar	Bhagwa	15.26	1.08	21.74	5.08
		Kustagi	Hiremonnapur	4	Mrigbahar	Bhagwa	31.24	3.53	31.39	22.76
			Modigere	4	Hastbahar	Bhagwa	20.26	1.44	-	-
			Shakapur	5	Mrigbahar	Bhagwa	56.78	4.21	40.28	35.76
			Yalabhurthi	4	Mrigbahar	Bhagwa	28.29	2.46	56.72	30.46

Sl. No.	District	Taluk	Village	Number of orchards	Cropping season	Variety	Leaf	Stem	Fruit	
							Per cent disease index	No. of lesions/ branch	Per cent incidence	Per cent disease index
		Yalburga	Hirearallalli	3	Mrigbahar	Bhagwa	15.27	1.06	22.74	30.58
			Makalli	3	Mrigbahar	Bhagwa	36.27	3.46	63.26	45.61
			Yadonni	6	Mrigbahar	Bhagwa	20.27	2.76	56.47	48.27
6.	Anantapur (AP)	Kanekal	L. B. Nagar	2	Ambiabahar	Ganesha	7.40	0.66	5.88	1.08
				3	Ambiabahar	Mrudula	7.03	2.40	37.68	22.22
			Rangapur Camp	4	Ambiabahar	Bhagwa	16.82	1.09	54.37	41.72
			Uddahal	4	Ambiabahar	Ganesha	18.27	2.63	26.43	15.71
		Rayadurga	Hiridal	4	Hastbahar	Bhagwa	21.39	3.06	41.59	25.67
			Hirehal	3	Mrigbahar	Ganesha	23.29	2.74	30.76	15.89
			Somalapur	2	Hastbahar	Ganesha	25.69	3.45	28.43	21.29
				3	Mrigbahar	Bhagwa	34.73	2.60	55.64	37.56

Note : Mrigbahar : Pruning from May-July
Hastbahar : Pruning from September-November
Ambiabahar : Pruning from February-April

Table 3: Taluk wise mean incidence and severity of bacterial blight of pomegranate in major areas of Karnataka and border areas of Andhra Pradesh over the years

Sl. No.	District	Taluk	Per cent disease index on leaf			Number of lesions on stem/branch			Per cent incidence on fruit			Per cent disease index on fruit		
			2006	2007	Average	2006	2007	Average	2006	2007	Average	2006	2007	Average
1.	Bagalkot	Bagalakot	25.37	22.74	24.06	1.60	1.54	1.57	33.79	25.38	29.59	25.66	16.56	21.12
2	Bellary	Bellary	15.86	12.13	13.99	1.95	2.02	1.99	32.80	23.63	28.22	16.15	10.19	13.17
		Hagaribommanahalli	23.19	13.49	18.34	2.69	2.54	2.62	40.35	32.04	36.20	23.54	21.54	22.54
3.	Bijapur	Bijapur	31.89	20.76	26.33	2.19	1.94	2.07	29.45	26.94	28.20	40.39	17.50	28.95
4.	Chitradurga	Hiriyur	28.06	12.22	20.14	2.01	1.28	1.64	55.54	27.33	41.44	38.99	18.66	28.83
		Mollakalmuru	19.66	22.79	21.23	1.76	1.95	1.86	27.27	42.99	35.13	19.94	31.68	25.81
5.	Koppal	Koppal	2.39	7.85	5.12	0.94	0.69	0.82	4.42	12.50	8.46	2.61	4.61	3.61
		Kustagi	24.58	35.14	29.86	3.01	2.91	2.96	51.36	42.80	47.08	33.46	29.66	31.56
		Yalburga	18.73	23.94	21.34	2.90	2.43	2.67	35.85	47.49	41.67	20.90	41.49	31.20
6.	Anantapur	Kannekal	18.73	12.38	15.56	1.39	1.70	1.55	44.59	31.09	37.84	28.59	20.18	24.39
		Rayadurga	21.64	26.28	23.96	3.09	2.96	3.03	31.74	39.11	35.43	19.66	25.10	22.38

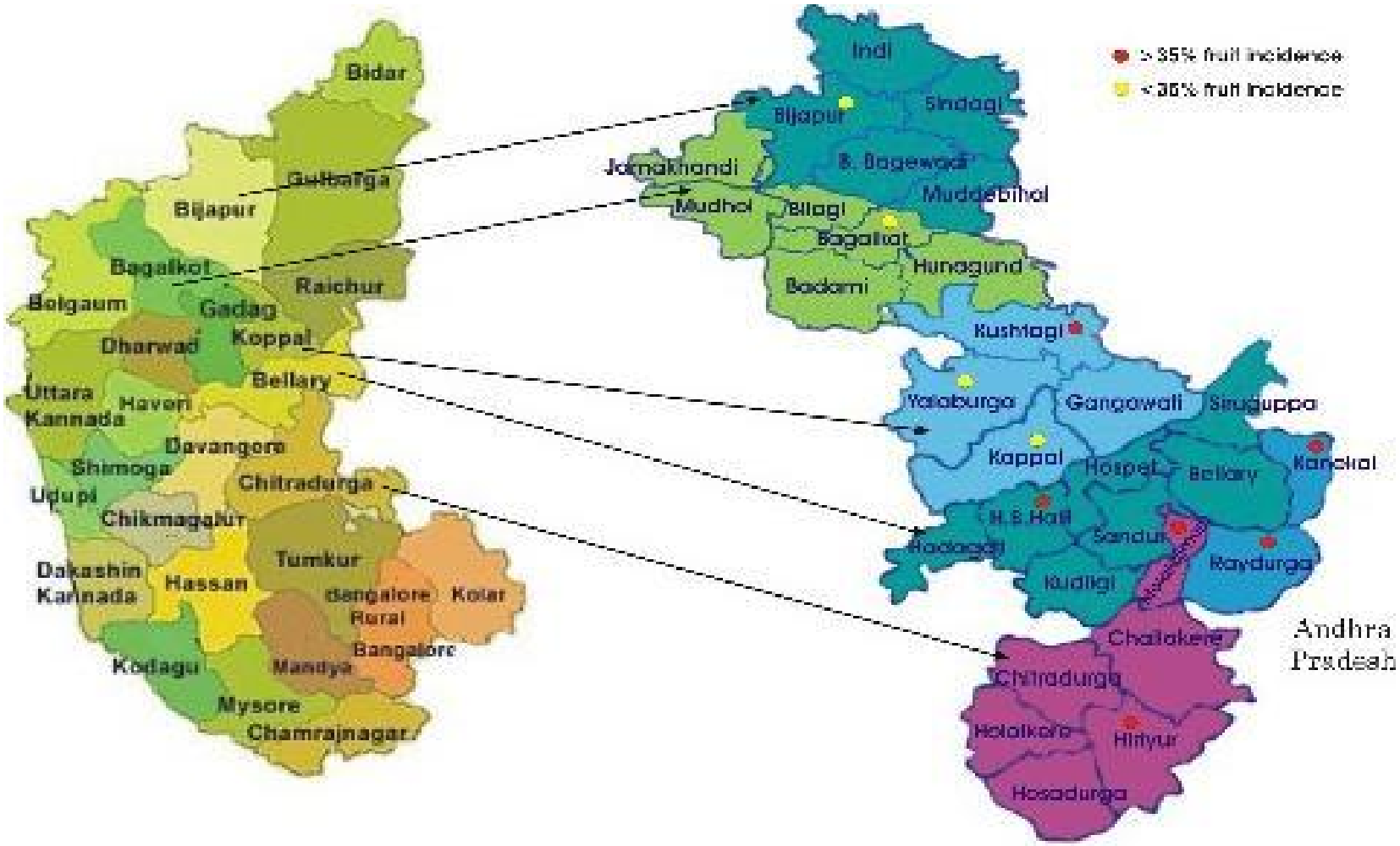
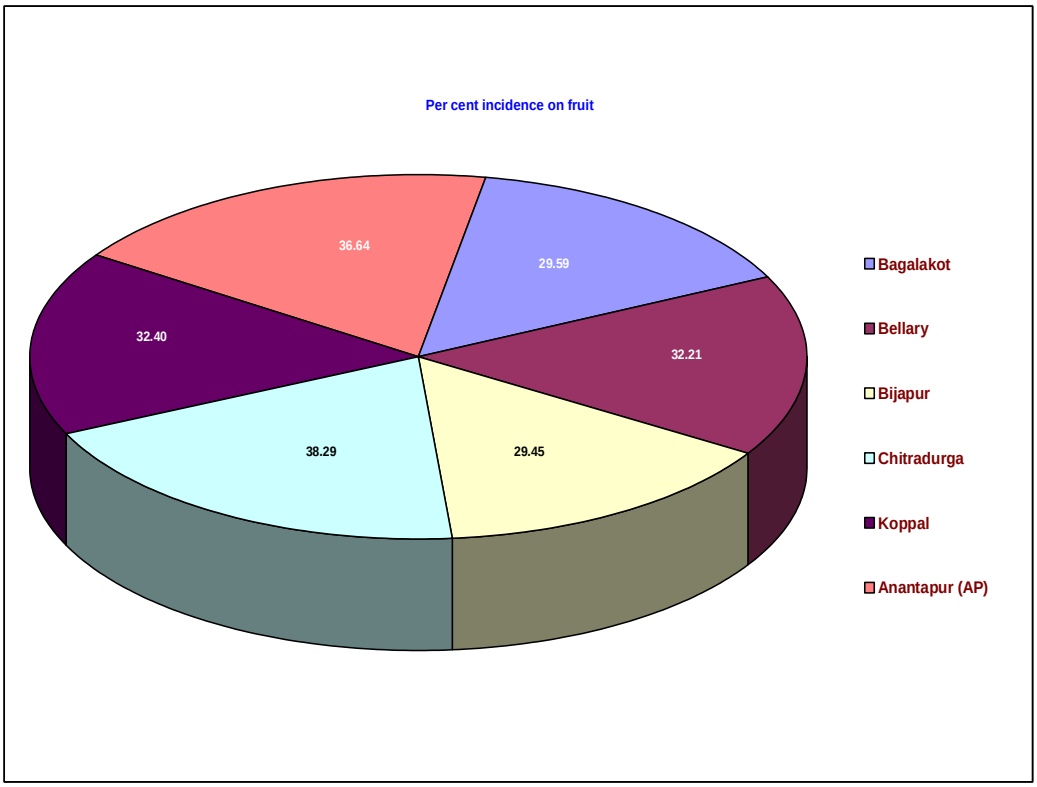


Fig.1: Survey on the incidence and severity of bacterial blight of pomegranate in major areas of Karnataka and border areas of Andhra Pradesh

Table 4: District wise mean incidence and severity of bacterial blight of pomegranate in major areas of Karnataka and border areas of Andhra Pradesh during 2006 and 2007

Sl. No.	District	Per cent disease index on leaf			Number of lesions on stem/ branch			Per cent incidence on fruit			Per cent disease index on fruit		
		2006	2007	Average	2006	2007	Average	2006	2007	Average	2006	2007	Average
1.	Bagalakot	25.37	22.74	24.06	1.60	1.54	1.57	33.79	25.38	29.59	25.68	16.56	21.12
2	Bellary	19.53	12.81	16.17	2.32	2.28	2.31	36.58	27.84	32.21	19.85	15.87	17.86
3.	Bijapur	31.89	20.76	26.33	2.19	1.94	2.07	29.45	26.94	29.45	40.39	17.50	28.95
4.	Chitradurga	23.86	17.51	20.69	1.89	1.62	1.76	41.41	35.16	38.29	29.47	25.17	27.32
5.	Koppal	15.23	22.31	18.77	2.29	2.01	2.15	30.54	34.26	32.40	18.99	25.25	22.12
6.	Anantapur (AP)	20.19	19.33	19.76	2.24	2.33	2.29	38.17	35.10	36.64	24.13	22.64	23.39



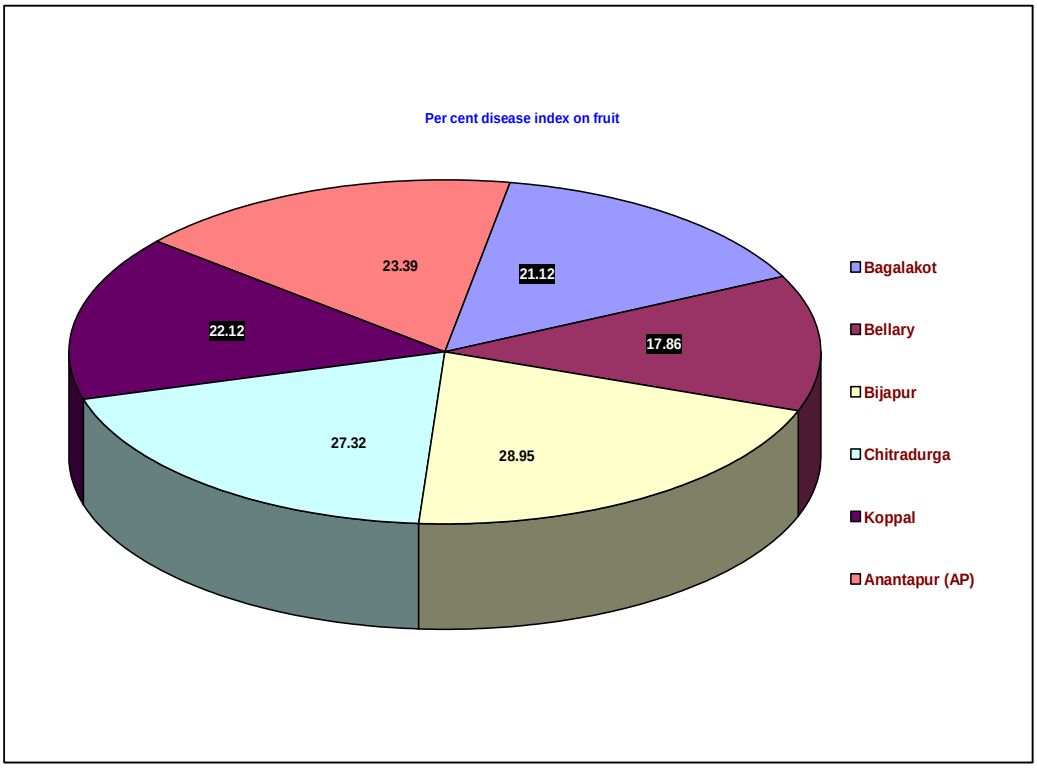


Fig. 2: District wise mean incidence and severity of bacterial blight of pomegranate on fruits surveyed over the seasons 2006 and 2007

Table 5a: Variety-wise mean incidence and severity of bacterial blight of pomegranate in major areas of Karnataka and border areas of Andhra Pradesh during 2006 and 2007

Season	Per cent disease index on leaf			Number of lesions on stem/branch			Per cent incidence on fruit			Per cent disease index on fruits		
	2006	2007	Average	2006	2007	Average	2006	2007	Average	2006	2007	Average
1. Bhagwa	20.92	19.18	20.05	2.10	2.06	2.08	33.29	32.14	32.72	24.32	21.09	22.71
2. Ganesha	19.61	16.48	18.05	1.52	1.67	1.60	24.70	22.48	23.59	17.33	12.84	15.09

Table 5b: Season-wise mean incidence and severity of bacterial blight of pomegranate in major areas of Karnataka and border areas of Andhra Pradesh during 2006 and 2007

Season	Per cent disease index on leaf			Number of lesions on stem/branch			Per cent incidence on fruit			Per cent disease index on fruits		
	2006	2007	Average	2006	2007	Average	2006	2007	Average	2006	2007	Average
1. Ambiabahar	19.10	13.84	16.47	2.83	1.83	2.33	43.74	25.82	34.78	27.43	18.61	23.02
2. Hastbahar	16.08	18.97	17.53	1.40	2.14	1.77	17.21	31.37	24.29	13.16	21.42	17.29
3. Mrigbahar	23.74	23.22	23.48	2.06	2.19	2.13	38.11	35.47	36.79	28.89	23.26	26.08

Disease was found quite severe on fruits in the plots surveyed at Rampura (Mollakalmura) village with maximum severity of 51.63 PDI on Bhagwa variety, followed by Shivanandanagar (50.84 PDI), Yadonni (48.27 PDI) and Makalli (45.61 PDI) villages during Mrigbahar on Bhagwa variety. Crop was found absolutely free from the disease in Irkalgada village of Koppal district.

District-wise intensity of bacterial blight on pomegranate surveyed during 2007 exhibited that (Table 3), maximum disease intensity of 22.74 PDI on leaf was observed in Bagalkot district followed by Koppal (22.31 PDI) and Bijapur (20.76 PDI) districts. Lowest leaf severity of 12.81 PDI was found in Bellary district. Number of lesions on the stem ranged between 1.54 to 2.28 per branch were observed in all the districts surveyed. Fruit infection ranged between 25.00 to 35.16 per cent with highest incidence of 35.16 per cent was observed in Anantapur district. Disease severity on the fruit observed during 2007 was quite less compared to 2006 and ranged from 15.87 to 25.25 PDI.

Taluk wise mean incidence over two years survey revealed that (Table 3), highest fruit incidence and severity were recorded in Kushtagi (per cent incidence of 47.08 and PDI of 31.56) and Yalburga (incidence of 41.67 per cent PDI of 31.20) taluks (Fig. 1) followed by Hiriyur. Lowest fruit incidence of 8.46 per cent and severity of 3.61 PDI were recorded in Koppal taluk.

Looking into the district average (Table 4), highest fruit infection of 38.29 per cent was recorded in Chitradurga district followed by Anantapur (36.64%), Koppal (32.40%) and Bellary (32.21%).

Correspondingly, average severity of the disease on fruits over two years ranged between 17.86 (Bellary) to 28.95 PDI (Bijapur) with moderate severity of 21 to 23 PDI recorded in Anantapur, Bagalkot and Koppal districts (Fig. 2).

Similarly, average leaf intensity of the disease ranged between 16.17 to 26.33 PDI with highest amount of disease on leaf in Bijapur district, whereas pomegranate plots in Bellary district indicated least disease index on leaf.

Among the varieties, Bhagwa occupied the larger cultivating area of pomegranate and was found highly susceptible to bacterial blight as evidenced by more disease severity recorded on leaf (20.05 PDI), stem (2.08 lesions/branch) and fruits (22.71 PDI) in all the areas surveyed (Table 5a). Ganesh variety was found.

With respect to the seasons, Mrigbahar was found highly favourable for the bacterial blight development (Table 5b) as more disease severity on leaf (23.48 PDI) and fruit (26.08 PDI) was recorded during the season. Disease pressure observed was low in Hastbahar and moderate in Ambiabahar.

4.2 Symptomatology

Symptoms were observed on leaf, stem and fruits. Initially small, water soaked, lesions were noticed on the lower surface of the leaves. Correspondingly on the upper surface small brown to black coloured spots were seen (Plate 1). Spots were round, angular to irregular in shape. As the disease progressed, these spots also grew, increased their size (2.0 – 5.0 mm in diameter), coalesced and extended upto midrib in a weeks time covering the major portion of the leaf lamina. Severely infected leaves turned yellow, became chlorotic and finally shed off.

Stem infection was manifested in the form of long, narrow and elongated light brown to black coloured lesions (1 – 4 cm long) on the main stem and branches. The lesions later on became rough and cankerous. As the disease advanced, stem girdling and breaking was seen at the point of infection. Symptoms were also noticed on flower buds in the form of small water soaked lesions, later on developed into brown to black coloured spots, leading to dropping of buds under severe cases. On developing green fruits, symptoms (Plate 2) were noticed as small, pin head sized, black lesions with diffused water soaked margin (oily spots), which later on develops into black coloured, medium to big sized spots (2 – 10 mm in diameter). One to many such spots were seen on the single fruit. Severely affected fruits split opens with L/Y/star shaped cracks within the spot. Infected fruits do not further develop nor suddenly drop, but dried up and remain hanged in the plant itself.

At early stage

At later stage

Leaf infection

Lesions at early stage

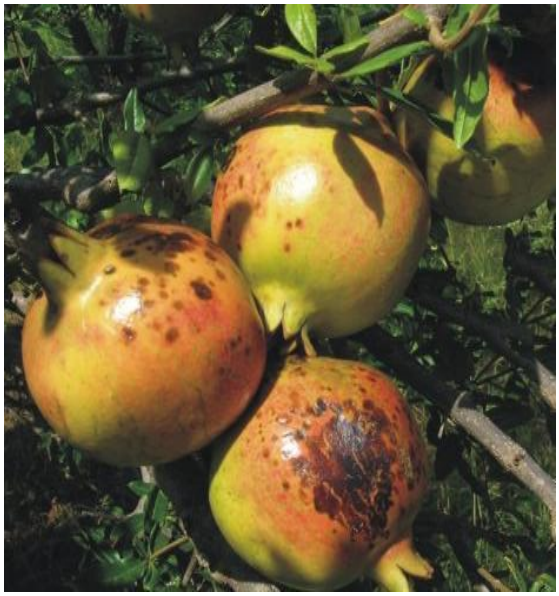
Lesions at later stage

Stem Infection

Symptoms on flower bud

Plate1: Symptoms of bacterial blight on leaf, stem and flower buds

At early stage



At later stage

Fruit infection

Disease severity on fruits

Fruit cracking due to disease severity

Plate 2: Symptoms of bacterial blight on fruits

4.3 Isolation of the pathogen and maintenance of pure culture

The causal organism was isolated from the infected leaf, bark of the stem and fruit pericarp showing typical symptoms of bacterial blight. Isolation was done by employing the serial dilution plating technique using nutrient agar medium. Repeated isolation from the infected plant parts yielded well separated, typical, yellow, mucoid, colonies of bacterium on nutrient agar medium after 72 hours of incubation at 30^0C (Plate 3). Colonies were purified by streaking the isolated colony on yeast-dextrose-calcium carbonate agar and pure colonies obtained were further streaked on to the nutrient agar slants and kept for incubation at 30^0C for 72 hours. Cultures so obtained were stored in the refrigerator at 5^0C, which served as a stock culture for further studies.

4.4 Pathogenicity

For proving pathogenicity, the bacterial cell suspension (5×10^6 cfu/ml) of each of the isolates collected from different regions during survey was sprayed on to the susceptible pomegranate plants of Bhagwa variety as described in "Material and Methods" chapter.

The characteristic symptoms were observed on pomegranate leaves after ten days of inoculation as 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 leading to marginal necrosis (Plate 3). Reisolations were carried out from these lesions for each isolate and comparisons were made with the original culture to confirm the identity of the pathogen. The isolates from artificially inoculated plants yielded the bacterial colonies similar to the original ones.

4.5 Identification of the pathogen

The identification of the pathogen involved in causation of bacterial blight of pomegranate was done by conducting studies on its morphological, biochemical, cultural and physiological features of the pathogen as per the standard microbiological procedures including host inoculation studies.

4.5.1 Morphological characters

The bacterium is rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative, capsulated, non-spore forming, having single polar flagellum and measured $0.4 – 0.25 \times 1.25 – 3.00$ μm in size. The cells readily stained with common stains such as crystal violet, gentian violet and carbol fuchsin (Plate 4).

4.5.2 Biochemical characters

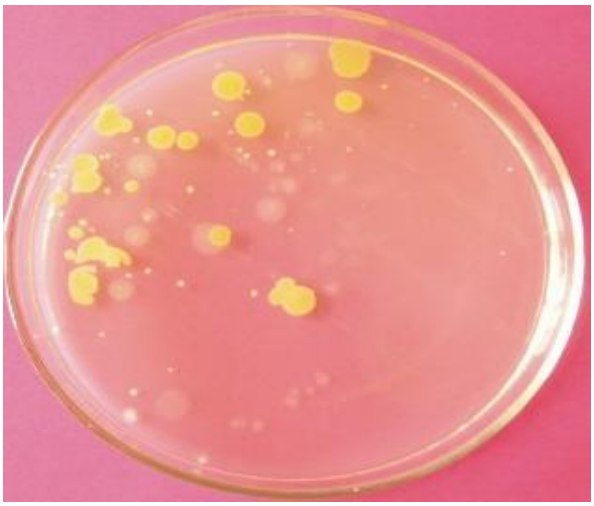
The results obtained on various biochemical characteristics of the pathogen are presented in Table 6, which revealed that, the bacterium liquefied the gelatin, hydrolysed the starch (Plate 5), positive for H_2S production, catalase and oxidase (Plate 6), utilized various carbon sources *viz.*, glucose, fructose, sucrose, dextrose and produced mild acid from these carbon sources, but did not utilize lactose, maltose, mannose and mannitol.

4.5.3 Cultural characters

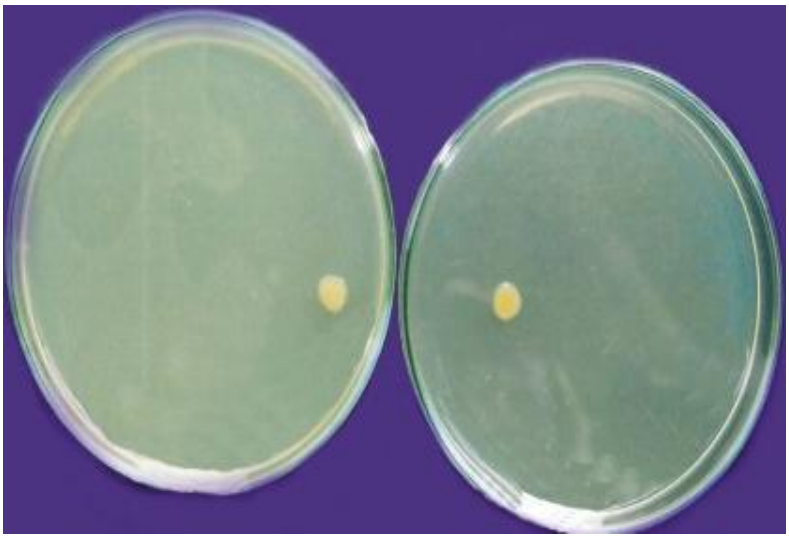
4.5.3.1 Efficacy of different media in supporting the growth of the pathogen

Of the various media tested for the efficacy to support the growth of *X. axonopodis* pv. *punicae*, modified D-5 medium (Table 7) was found significantly superior in promoting the luxurious growth of the pathogen as evidenced by the maximum recovery of bacterial colonies (150.75×10^5 cfu/ml) followed by yeast extract nutrient agar medium, which gave 110.75×10^5 cfu per ml. These two media differed significantly with each other and also superior to other tested media. The next best media for the cultivation of pomegranate bacterium were GYCA (67.50×10^5 cfu/ml), Tween 80 (61.50×10^5 cfu/ml) and nutrient agar (60.75×10^5 cfu/ml), which exhibited on par efficacy with each other in respect of promoting the growth and development of bacterial colonies.

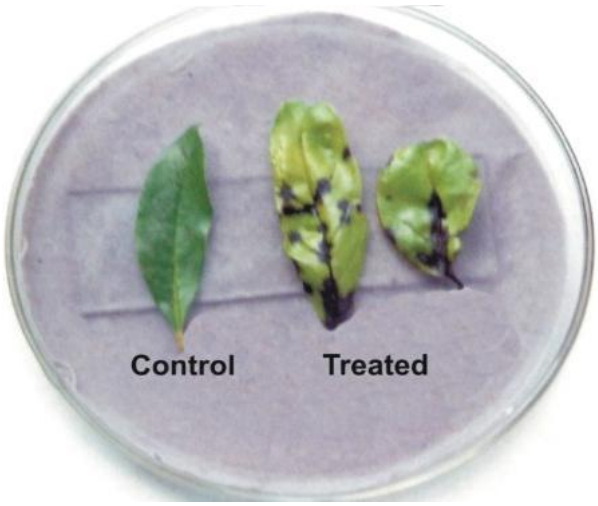
The bacterium grew moderately on other media such as XTS, starch and YDCA in which number of colonies ranged between 55×10^5 to 40.25×10^5 cfu per ml. Significantly



Colonies of X. axonopodis pv.punicae on nutrient agar medium

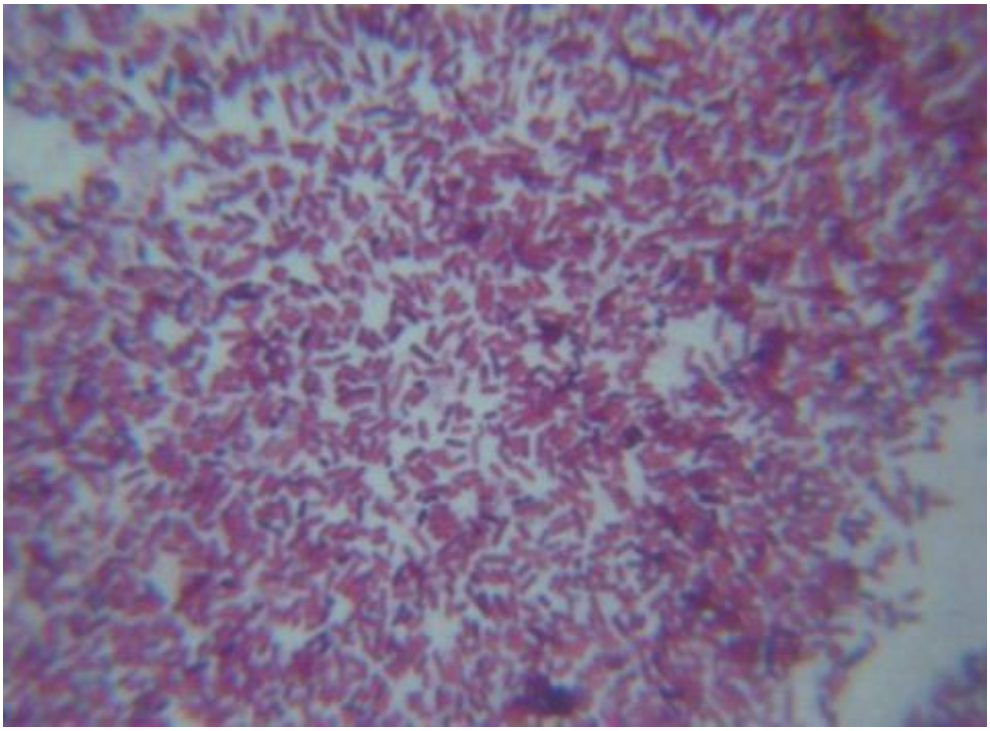


Isolated colony of X. axonopodis pv.punicae on nutrient agar medium

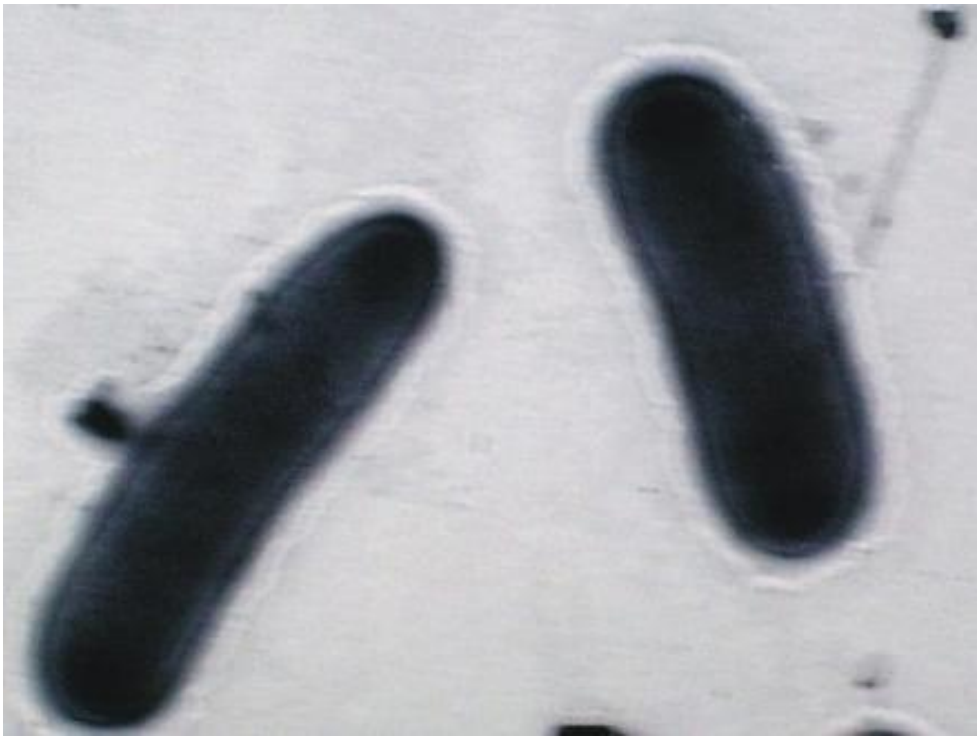


Pathogenicity on leaf

Plate 3: Isolation and pathogenicity



Gram stained cells of X.axonopodis pv. punicae (1000x)



Scanning electron microphotograph of X. axonopodis pv. punicae($4x10^5$)

Plate 4: Morphology of the bacterium

Table 6: Biochemical characteristics of *Xanthomonas axonopodis* pv. *punicae*

Sl. No.	Characteristics	Reaction
1.	Starch hydrolysis	+
2.	Gelatin liquefaction	+
3.	H_2S production	+
4.	Catalase	+
5.	Oxidase	+
6.	Acids from	
a)	Glucose	+
b)	Fructose	+
c)	Lactose	-
d)	Maltose	-
e)	Sucrose	+
f)	Dextrose	+
g)	Mannose	-
h)	Mannitol	-

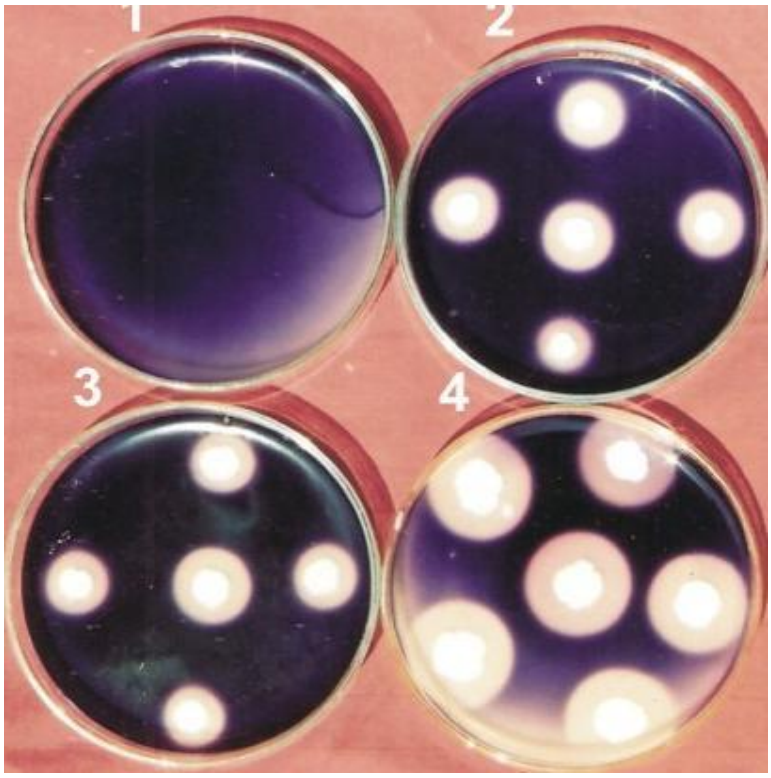
least number of colonies (10×10^5 cfu/ml) were recorded in BSCAA medium and no growth of the bacterium was observed on SX agar (Fig. 3).

Similarly, colony diameter on various media ranged from 0.5 to 4.0 mm and comparatively medium to large sized colonies (2 – 4.0 mm and above) were recorded on YNA, YDCA, GYCA and MD-5 medium. Colony diameter was very small (0.5 – 1.0 mm) in XTS agar.

4.5.3.2 Colony morphology

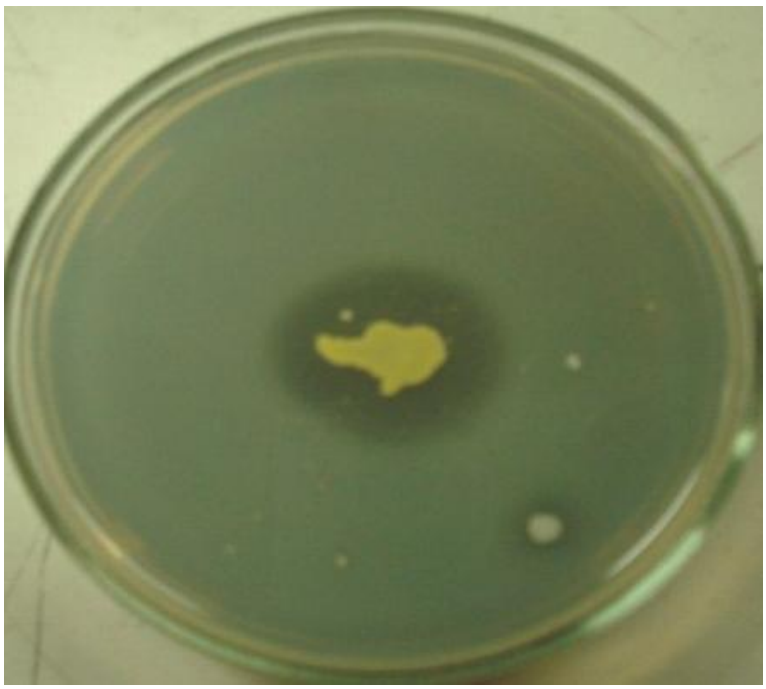
Colonies of the bacterium on MD-5 and YNA (Table 7) medium appeared as circular to irregular, flattened, colourless to light yellow, occurred singly or rarely in aggregate (Plate 7). Colonies of similar morphology with glistening character and bright yellow colour were observed on both GYCA and YDC medium. Circular to irregular, slightly raised, mucoid colonies were recorded on nutrient agar and starch agar medium.

XTS agar supported the moderate growth of the bacterium with minute, slightly raised, circular, creamy white coloured colonies. Bacterium exhibited very poor growth with dull white and slightly raised colonies character on BSCAA medium and was found failed to grow on SX agar.



1. Untreated control
2.Bijapur isolate
3. Bagalkot isolate
4. Bellary isolate

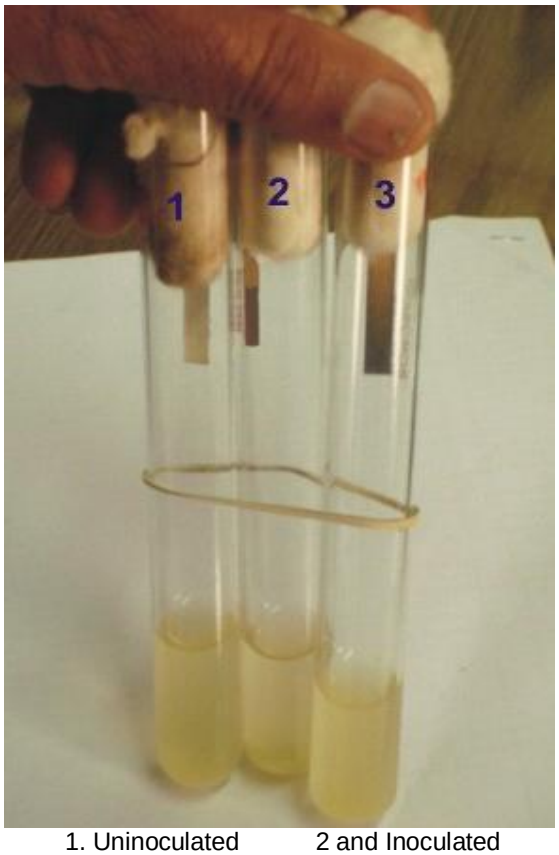
Starch hydrolysis



Gelatin liquefaction

Plate 5: Biochemical characters of X. axonopodis pv.punicae

Oxidase activity



1. Uninoculated 2 and Inoculated
H_2S production

Plate6: Biochemical characters of X.axonopodis pv.punicae

Table 7: Growth and cultural characteristics of *Xanthomonas axonopodis* pv. *punicae* on different solid media

Sl. No.	Media	Colonies of the bacterium (10^5 cfu/ml)	Size of the colony (Range mm)	Average of size of the colony (mm)	Colony characters		
					Colour	Shape	Appearance
1.	BSCAA	10.00 (3.31)*	1 – 2.5	1.72	Dull white	Circular to irregular	Slightly raised and convex
2.	GYCA	67.50 (8.27)	2 – 4	2.99	Yellow	Circular to irregular	Flattened, glistening
3.	NA	60.75 (7.85)	1-2	1.63	Light yellow to yellow	Mostly circular	Convex, mucoid, slimy, glistening
4.	Modified D-5	150.75 (12.31)*	2-4	2.75	Light yellow to light brown	Circular to irregular	Flattened
5.	Starch	52.00 (7.27)	2 – 3	2.81	Light yellow to yellow	Circular to irregular	Slightly raised, slimy glistening
6.	SX	0.00 (1.00)	-	-	-	-	-
7.	Tween 80 medium	61.50 (7.90)	1 – 2	1.43	Light yellow	Circular to irregular	Convex
8.	YDCA	40.25 (6.41)	2 – 4.0	2.56	Bright yellow	Mostly circular	Flattened / slightly raised, glistening
9.	YNA	110.75 (10.56)	2 – 4.0	2.72	Pale yellow	Circular to irregular	Flattened
10.	XTS agar	55.00 (7.47)	0.5 – 1	0.70	Creamy white to light yellow	Circular	Slightly raised
	SEm±	0.22	-				
	CD at 1%	0.85					

* - $\sqrt{x+1}$ transformed values

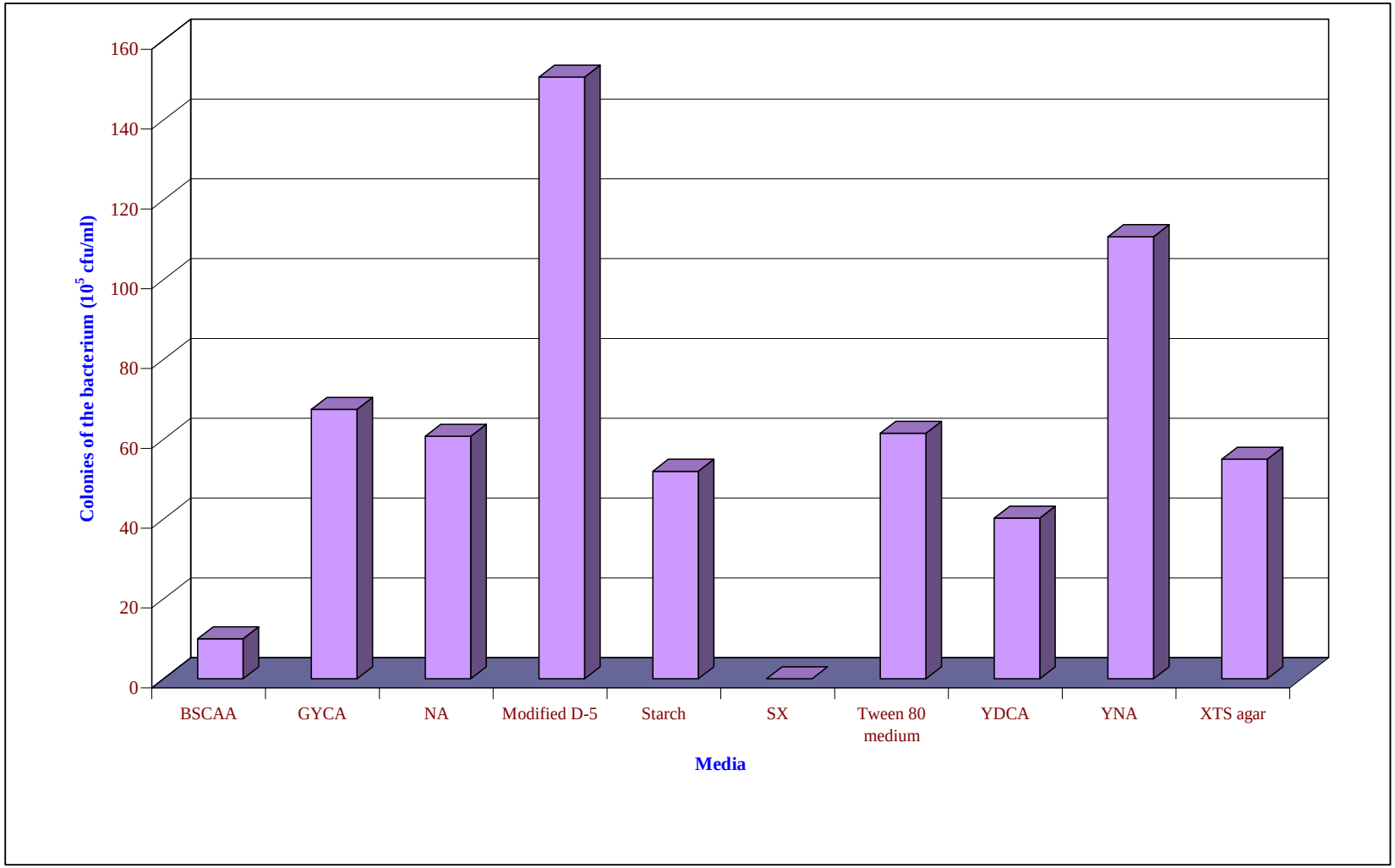


Fig. 3: Growth of Xanthomonas axonopodis pv. punicae on different solid media

4.6 Cultural variability

4.6.1 Growth of isolates in different media

Cultural variability of 20 isolates of the pathogen collected from various regions during survey was studied as per the procedure given in Material and Methods.

The results of the study indicated that, all the five media tested for variability differed significantly with each other in supporting the growth of different isolates of the pathogen (Table 8) with maximum growth (number of colonies) of isolates recorded in modified D-5 medium (89.56×10^5 cfu/ml) followed by Tween 80 (46.32×10^5 cfu/ml) and XTS (38.77×10^5 cfu/ml). Significantly less number of isolates were grown on SX agar with average colonies of 0.70×10^5 cfu per ml.

Comparison among the different isolates on six media revealed that, modified D-5 medium was significantly superior (Fig. 4) in supporting the maximum growth of Xa7 (165.33×10^5 cfu/ml), which was on par with Xa17 (156×10^5 cfu/ml), Xa16 (142.33×10^5 cfu/ml) and Xa19 (139×10^5 cfu/ml). The next highest colony number ranged between 82 – 107.63×10^5 cfu per ml were recorded by Xa10, Xa18, Xa14, Xa4, Xa1 and Xa12 and are on par with each other. The isolate Xa8 recorded least number of colonies (44.66×10^5 cfu/ml).

The next best medium was Tween 80, which supported the maximum growth of Xa14 (90.67×10^5 cfu/ml), Xa7 (75.33×10^5 cfu/ml), Xa20 (74×10^5 cfu/ml) and Xa1 (67×10^5 cfu/ml, Xa11 (64.33×10^5 cfu/ml) isolates. The least growth was recorded by Xa12 (11.67×10^5 cfu/ml) isolate.

The isolate which recorded significantly highest growth on XTS agar was Xa11 (83.67×10^5 cfu/ml) followed by Xa10, Xa2, Xa20, Xa17, Xa15, Xa9, Xa7, Xa16 and Xa3 isolates, which recorded on par number of colonies ranged between 38 - 58×10^5 cfu per ml.

The isolate Xa5 recorded the least number of colonies (5.33×10^5 cfu per ml).

In general, the isolates did not grow well on BSCAA medium. However, the isolates Xa12 (26.33×10^5 cfu/ml), Xa13 (19×10^5 cfu/ml), Xa16 (18.33×10^5 cfu/ml), Xa10 (17.67×10^5 cfu/ml), Xa18 (17.66×10^5 cfu/ml), Xa15 (17.00×10^5 cfu/ml), Xa20 (16.67×10^5 cfu/ml) and Xa5 (16.00×10^5 cfu/ml) showed on par growth with each other, which was significantly higher than rest of the isolates. The lowest growth was recorded by Xa8 (2.60×10^5 cfu/ml) and the isolates Xa3 and Xa4 fail to grow on BSCAA medium.

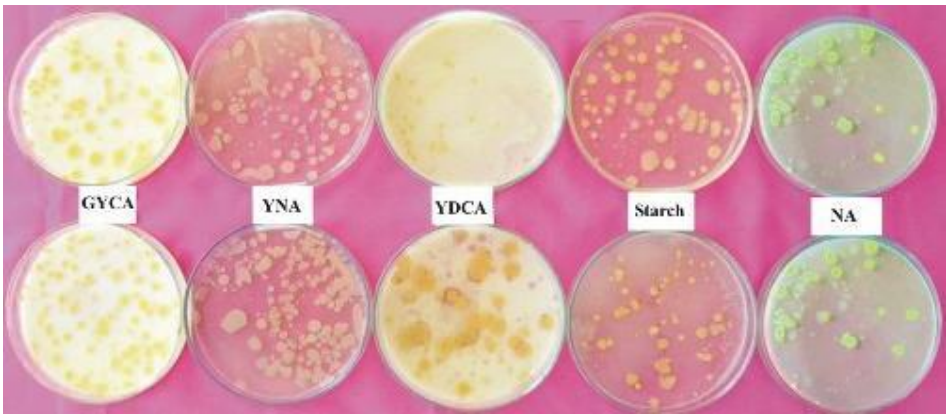
Similarly, SX agar failed to support the growth of 15 isolates, although five isolates *viz.*, Xa3, Xa6, Xa7, Xa10 and Xa14 recorded the growth ranged between 1.33 to 4.33×10^5 cfu per ml and were on par with each other.

The comparison between the growth of isolates irrespective of media revealed that, Xa7 significantly recorded the highest number of colonies (61.87×10^5 cfu/ml) and was on par with Xa17, Xa16, Xa11, Xa19, Xa20, Xa14, Xa10, Xa15, Xa1 and Xa9. The colonies of these isolates ranged between 36.67 to 55.73×10^5 cfu per ml. All other isolates grew moderately with on par colony number ranged between 19.27 to 33.86×10^5 cfu per ml.

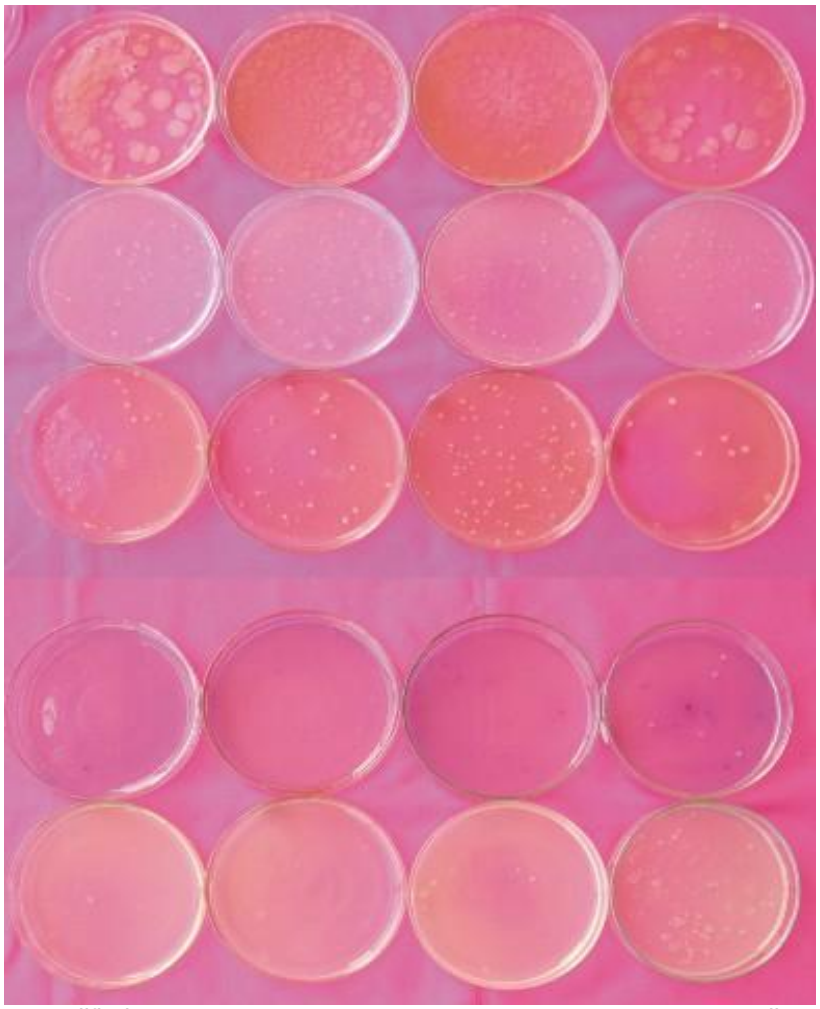
4.6.2 Variability on colony morphology

The behaviour of different isolates of pomegranate bacterium on five different media presented in Table 9, revealed the variation in respect of colony characters such as size, shape, colour and appearance.

On modified D-5 medium, the isolates differed greatly in respect of colony colour as the isolates Xa3, Xa4, Xa5, Xa7, Xa14 and Xa16 exhibited creamy white colonies, while, light yellow colonies were exhibited by Xa1, Xa6, Xa8, Xa12 and Xa15 isolates. The isolates Xa2, Xa10, Xa17 and Xa19 produced light brown coloured colonies. Variation in size and shape on the similar medium revealed that the colonies of Xa1, Xa7, Xa10, Xa11, Xa12, Xa17, Xa18 and Xa20 produced small to medium colonies with diameter ranged between 1.0 to 2.0 mm (small) to 2.1 to 3.0 mm (medium) and were circular to irregular in shape. However, the colonies of Xa2, Xa5 and Xa9 were very small (<1.0 mm), whereas the isolate Xa15 produced large sized colonies (3.1 – 4.0 mm). The appearance of colonies in different isolates was



a. Colonies of X. axonopodis pv.punicae on differential media



1. Modified D-5 2. XTS agar 3. Tween-80 medium
4. SX agar 5. BSCAA medium

b.Colonies of X. axonopodis pv. punicae on selective/semi-selective medium

Plate 7: Cultural studies

Table 8: Variability in colony number of twenty isolates of *Xanthomonas axonopodis pv. punicae* on five selective/semi-selective media

Isolates	Colonies of the bacterium (10^5 cfu/ml) on different media					
	Modified D-5	Tween 80	XTS	SX	BSCAA	Mean
Xa1	97.33 (9.88)*	67.00 (8.22)	16.67 (4.18)	0.00 (1.00)	6.00 (2.63)	37.40 (5.18)
Xa2	45.67 (6.81)	27.66 (5.32)	58.00 (7.65)	0.00 (1.00)	4.00 (2.23)	27.07 (4.60)
Xa3	70.67 (8.43)	19.33 (4.49)	38.00 (6.25)	1.60 (1.62)	0.00 (1.00)	25.92 (4.36)
Xa4	95.32 (9.80)	14.67 (3.94)	11.67 (3.53)	0.00 (1.00)	0.00 (1.00)	24.33 (3.85)
Xa5	75.00 (8.71)	54.33 (7.42)	5.33 (2.46)	0.00 (1.00)	16.00 (4.08)	30.13 (4.73)
Xa6	53.66 (7.38)	24.67 (5.03)	9.00 (2.86)	1.33 (1.47)	7.67 (2.94)	19.27 (3.94)
Xa7	165.33 (12.89)	75.33 (8.73)	55.00 (7.39)	3.67 (2.14)	10.00 (2.99)	61.87 (6.83)
Xa8	44.66 (6.74)	36.00 (6.04)	23.67 (4.91)	0.00 (1.00)	2.60 (1.82)	21.39 (4.10)
Xa9	56.00 (7.54)	64.00 (8.05)	54.33 (7.43)	0.00 (1.00)	9.00 (3.14)	36.67 (5.43)
Xa10	82.00 (9.10)	33.67 (5.86)	62.67 (7.96)	4.33 (2.30)	17.67 (4.30)	40.07 (5.90)
Xa11	75.66 (8.74)	64.33 (8.05)	83.67 (9.19)	0.00 (1.00)	12.33 (3.63)	47.20 (6.12)
Xa12	107.63 (10.40)	11.67 (3.54)	23.66 (4.93)	0.00 (1.00)	26.33 (5.20)	33.83 (5.01)
Xa13	55.66 (7.52)	34.00 (5.88)	36.00 (6.07)	0.00 (1.00)	19.00 (4.46)	28.93 (4.98)
Xa14	94.33 (9.75)	90.67 (9.53)	24.33 (5.01)	3.00 (1.99)	4.60 (1.88)	43.39 (5.63)
Xa15	74.33 (8.67)	43.33 (6.64)	55.67 (7.47)	0.00 (1.00)	17.00 (4.24)	38.07 (5.60)
Xa16	142.33 (11.96)	56.67 (7.58)	43.33 (6.64)	0.00 (1.00)	18.33 (4.37)	52.13 (6.31)
Xa17	156.00 (12.53)	62.33 (7.93)	56.33 (7.57)	0.00 (1.00)	4.00 (2.24)	55.73 (6.26)
Xa18	84.67 (9.25)	18.00 (4.34)	32.33 (5.74)	0.00 (1.00)	17.66 (4.27)	30.53 (4.92)
Xa19	139.00 (11.82)	54.67 (7.46)	29.00 (5.39)	0.00 (1.00)	10.67 (3.40)	46.67 (5.80)
Xa20	76.00 (8.75)	74.00 (8.65)	56.67 (7.57)	0.00 (1.00)	16.67 (4.16)	44.67 (6.03)
Mean	89.56 (9.33)	46.32 (6.63)	38.77 (6.07)	0.70 (1.23)	10.98 (3.16)	37.27 (5.28)

Source	SEm±	CD at 1%
Isolates	0.18	0.65
Media	0.09	0.32
Isolates × Media	0.40	1.45

$\sqrt{\ }$ ×+1 transformed values

Table 9: Cultural characteristics of different isolates of *Xanthomonas axonopodis* pv. *punicae* on different media

Media	Colony characters	Xa1	Xa2	Xa3	Xa4	Xa5	Xa6	Xa7	Xa8	Xa9	Xa10
BSCAA	Colour	Light yellow	Dull white	Light yellow	Light yellow	Dull white to light yellow	Dull white	Creamy white to light yellow	Dull white	Dull white	Dull white to light yellow
	Size	Very small	Small	Small	Very small to small	Small	Very small	Small	Very small	Small	Small
	Shape	Highly circular	Circular	Circular	Circular to irregular	Circular	Circular to irregular	Circular to irregular	Circular	Circular	Circular
	Appearance	Convex	Slightly raised	Convex	Slightly raised	Convex slightly raised	Slightly raised	Flattened	Convex, slightly glistening	Convex, glistening	Convex, glistening
Modified D-5 medium	Colour	Light yellow to brown	Light brown	Creamy white	Creamy white	Creamy white	Light yellow	Creamy white to light brown	Pale yellow	Creamy white to light yellow	Light brown
	Size	Small to medium	Small	Medium to large	Medium	Small	Medium	Small to medium	Medium	Small	Small to medium
	Shape	Irregular	Irregular	Circular to irregular	Mostly circular	Circular	Circular	Circular to irregular	Circular	Mostly circular	Circular
	Appearance	Flattened	Slightly raised	Smooth, flattened	Flattened	Slightly raised, glistening	Convex, slimy	Flattened, slightly glistening	Convex, glistening	Convex slimy	Flattened
SX	Colour		Dull white to slightly blue				Light yellow	White with blue centre			Dull white to slightly blue
	Size	No growth	Medium	No growth	No growth	No growth	Small	Small	No growth	No growth	Small
	Shape		Irregular				Circular	Circular			Circular
	Appearance		Convex				Flattened	Flattened			Flattened
Tween 80	Colour	Colourless to pale yellow	Pale yellow	Yellow	Pale brown	Yellow	Light yellow to slightly brown	Yellow	Creamy white	Light yellow	Pale yellow
	Size	Small to medium	Small	Small	Medium	Small	Medium	Small to medium	Small	Small	Medium
	Shape	Circular	Circular	Circular to irregular	Irregular	Mostly circular	Circular to irregular	Circular	Circular to irregular	Circular	Circular
	Appearance	Convex, glistening	Slightly raised	Convex	Slightly raised, slimy glistening	Convex	Flattened	Flattened	Flattened	Convex glistening	Convex glistening
XTS	Colour	Creamy white	Light yellow	Yellow	Dull white to pale yellow	Creamy white	Yellow	Yellow	Yellow	Yellow to dark yellow	Creamy white
	Size	Small	Small	Small to medium	Very small	Small	Small	Small	Small	Very small	Very small
	Shape	Circular	Circular	Mostly irregular	Circular	Circular to irregular	Circular	Circular	Circular to irregular	Circular	Circular
	Appearance	Slightly raised convex	Convex	Slightly raised mucoid, glistening	Slightly flattened	Convex	Flattened, slightly glistening	Flattened, slightly glistening	Convex, glistening	Flattened, glistening	Convex

Contd…….

Media	Colony characters	Xa11	Xa12	Xa13	Xa14	Xa15	Xa16	Xa17	Xa18	Xa19	Xa20
BSCAA	Colour	Creamy white	Light yellow	Dull white	Creamy white to light yellow	Light yellow	Creamy white	Pale yellow	Yellow	Creamy white to light yellow	Dull white
	Size	Small	Very small to small	Small	Small to medium	Small	Very small to small	Small	Small	Small	Small
	Shape	Circular	Mostly irregular	Circular	Mostly circular	Circular to irregular	Circular to slightly raised	Circular slightly raised, glistening	Circular	Irregular	Circular to irregular
	Appearance	Convex, glistening	Convex	Convex, glistening	Slightly raised	Convex	Convex		Slightly raised, glistening	Convex	Convex
Modified D-5 medium	Colour	Creamy white	Light yellow	Creamy white to light brown	Creamy white	Light yellow	Creamy white	Light brown	Creamy white	Light brown	Creamy white
	Size	Small to medium	Small to medium	Medium	Medium	Large	Medium	Small to medium	Small to medium	Medium to large	Small to medium
	Shape	Circular	Irregular	Circular	Circular to irregular	Circular	Mostly irregular	Circular to irregular	Circular	Circular to irregular	Mostly circular
	Appearance	Flattened	Slightly raised glistening	Flattened, slimy	Flattened	Convex, slimy, glistening	Flattened	Flattened	Slightly raised, glistening	Convex, glistening	Flattened
SX	Colour				Slightly blue						
	Size	No growth	No growth	No growth	Small	No growth	No growth	No growth	No growth	No growth	No growth
	Shape				Circular to irregular						
	Appearance				Convex						
Tween 80	Colour	Light yellow	Pale yellow	Dull white	Creamy white	Creamy white	Light yellow	Pale brown	Pale yellow, light brown	Light yellow	Light yellow
	Size	Small	Medium	Small to medium	Medium	Small	Small	Medium	Small to medium	Medium	Small
	Shape	Circular to irregular	Circular to irregular	Mostly irregular	Mostly circular	Circular to irregular	Circular	Circular to irregular	Mostly irregular	Mostly circular	Circular
	Appearance	Convex, glistening	Convex	Slightly raised	Flattened	Flattened	Flattened, slightly, glistening	Slightly raised	Slightly raised	Convex, glistening	Flattened
XTS	Colour	Creamy white to light yellow	Dull white/ creamy white	Yellow	Yellow	Creamy white to light yellow	Light yellow	Pale yellow	Creamy white	Yellow	Dull white to creamy white
	Size	Very small to small	Small to medium	Small	Very small	Very small to small	Very small to small	Small	Very small to small	Small	Very small to small
	Shape	Irregular	Circular to irregular	Irregular	Mostly circular	Irregular	Circular to irregular	Irregular	Irregular	Circular to irregular	Irregular
	Appearance	Convex	Convex	Slightly raised, glistening	Convex	Slightly raised	Slightly raised	Convex	Slightly raised	Convex	Convex

Very small : <1.0 mm; Small : 1-2 mm; Medium : 2.1-3.0 mm; Large : 3.1-4.0 mm

either flattened, slightly raised or convex with or without glistening character. The maximum number of isolates produced flattened colonies as against mucoid, slightly glistening and convex colonies of isolates Xa6, Xa8, Xa15 and Xa19. .

The colony characters of twenty isolates differed on Tween 80 medium. The colonies were yellow (Xa3, Xa5 and Xa7), pale yellow (Xa2, Xa10, Xa11, Xa12) and creamy white (Xa8, Xa14, Xa15) in ten isolates. The size of the colony was small in 14 isolates, whereas medium sized colonies were observed in Xa4, Xa6, Xa10, Xa12, Xa14 and Xa17 isolates. The shape was circular in maximum number of isolates, however, irregular shaped colonies were produced by Xa6 and Xa18 isolates. The colonies were slightly raised or convex, mucoid and glistening in case of Xa3, Xa12, Xa18, Xa19. The isolates Xa6, Xa7, Xa8, Xa14 and Xa15 possessed flattened colonies.

On XTS agar medium, very small, creamy white, circular to irregular colonies were produced by isolates *viz.*, Xa6, Xa10, Xa11, Xa12, Xa15. The isolates Xa3, Xa6, Xa7, Xa8, Xa13, Xa14 and Xa19 produced small and yellow coloured colonies. The appearance was slightly raised (Xa1, Xa3, Xa6) or convex (Xa8, Xa10, Xa11, Xa12) in seventeen isolates. Only two isolates Xa7 and Xa9 showed flattened, glistening colonies.

Fifteen isolates fail to grow on SX agar. However, the isolates Xa2, Xa6, Xa7, Xa10 and Xa14 produced small, circular, flattened, dull white (with slightly blue colour at the centre) colonies with an exception to this the isolate Xa2 produced medium sized, irregular and convex colonies.

On BSCAA medium, colonies of most of the isolates differed from other isolates as small, dull white and circular (Xa2, Xa5, Xa9, Xa11, Xa13), whereas colonies of Xa1, Xa4 and Xa12 isolates appeared as very small with light yellow in colour. The appearance being convex in thirteen isolates. Slightly raised and glistening colonies were observed in Xa2, Xa4, Xa6, Xa14, Xa16 and Xa18 isolates. None of the isolates found to have flattened colonies on BSCAA medium.

4.6.3 Morphological groupings

Morphological groupings (Table 10) were made among the isolates of pomegranate bacterium based on the colony characters on modified D-5 medium. A total of five groups were made. Of them G-II and G-I were predominant with highest frequency of 35 and 30 per cent, respectively (Fig. 5). Group-I included Xa1, Xa2, Xa7, Xa10, Xa12 and Xa17 isolates and colonies of these isolates were small to medium sized, circular to irregular, flattened or slightly raised with light yellow to light brown in colour. Group-II was the biggest one with seven isolates having medium sized, circular to irregular, flattened and creamy white colonies.

The colonies of isolates belonging to G-III were slightly raised or convex, circular, small to medium sized, creamy white to light yellow in colour with glistening character.

G-IV and G-V were the smallest groups each having two number of isolates differed in respect of colony colour and morphology. G-IV isolates exhibited circular to medium sized, light yellow coloured colonies. The colonies of G-V isolates were found medium to large sized, circular to irregular, light yellow to light brown in colour. Further, the colonies of both the group isolates were convex, slimy and glistening in appearance.

4.6.4 Molecular variability

To distinguish within a species using traditional morphological differences through cultural studies is often incomplete and misleading. So, the molecular technique such as Random Amplified Polymorphic DNA (RAPD) was employed to detect the variations among the 20 isolates of *X. axonopodis* pv. *punicae* causing bacterial blight of pomegranate. A total of 20 primers falling in OPA, OPB and OPF series as mentioned in “Material and Methods” were used to determine the genetic distance between the isolates and to construct a dendrogram. Banding profile of 11 primers for 20 isolates of *X. axonopodis* pv. *punicae* are furnished in Table 11.

Results on differentiation of isolates revealed that, out of 20 primers used, 11 primers resulted the amplification, among which, the primers OPA-20, OPB-03, OPF-07 and OPF-10 exhibited 100 per cent polymorphism (Plate 8).

Table 10: Morphological groupings of 20 isolates of *Xanthomonas axonopodis* pv. *punicae* based on cultural characteristics on MD-5 medium

Group	No. of isolates	Colony characters	Frequency
I	Xa1, Xa2, Xa7, Xa10, Xa12, Xa17	Light yellow to light brown; small to medium sized; circular to irregular; flattened or slightly raised colonies	30%
II	Xa3, Xa4, Xa11, Xa13, Xa14, Xa16, Xa20	Creamy white; medium sized; circular to irregular; flattened colonies	35%
III	Xa5, Xa9, Xa18	Creamy white to light yellow coloured; small to medium sized; mostly circular; slightly raised or convex glistening colonies	15%
IV	Xa6, Xa8	Light yellow; medium sized; circular; convex, slimy with glistening character	10%
V	Xa19, Xa15	Light yellow to light brown; medium to large; circular to irregular; convex and glistening colonies	10%

Out of 60 amplicon levels resulted from 11 primers used for analysis, 51 amplicons were found polymorphic. On an average, there were 5.5 amplicon levels per primer of which 4.5 were polymorphic, indicating that, there was a significant molecular variability between the different isolates of pomegranate pathogen subjected for molecular analysis. The information pertaining to the banding pattern for all the primers was used to determine genetic differentiation among the isolates and to construct a dendrogram.

Based on simple matching coefficient a genetic similarity matrix was constructed to assess the relatedness among the isolates of pomegranate bacterial pathogen. Genetic similarity coefficient of 20 isolates based on RAPD analysis (Table 12) ranged from 0.47 to 0.81.

The highest genetic similarity to an extent of 81 per cent was recorded between Xa3 and Xa6 isolates followed by 80 per cent similarity between Xa9 and Xa19 isolates. Least genetic similarity was observed between Xa12 and Xa1, Xa15 and Xa1 (47%) isolates. Further, the dendrogram (Fig. 6) constructed from the pooled data indicated that, there were two major clusters A and B at similarity coefficient of 0.60. Cluster A was further sub-grouped into 28 sub-clusters representing different isolates, while cluster B consisted of a single sub-cluster, representing Xa1 (S. J. Kote - Bellary) and xa18 (Tikota – Bijapur) isolates.

4.7 Physiological characters

4.7.1 Temperature requirement

Optimum temperature requirement for the growth of *X. axonopodis* pv. *punicae* was studied as per the procedure narrated in “Material and Methods”. The results are presented in Table 13.

The effect of varied temperature levels on the growth of *Xanthomonas axonopodis* pv. *punicae* was significant. The temperature of 30^0C was found cardial for the growth of pathogen as significantly maximum number of colonies (147.33×10^5 cfu/ml) were recovered at this temperature level followed by 25^0C (134×10^5 cfu/ml) and 35^0C (83×10^5 cfu/ml). The

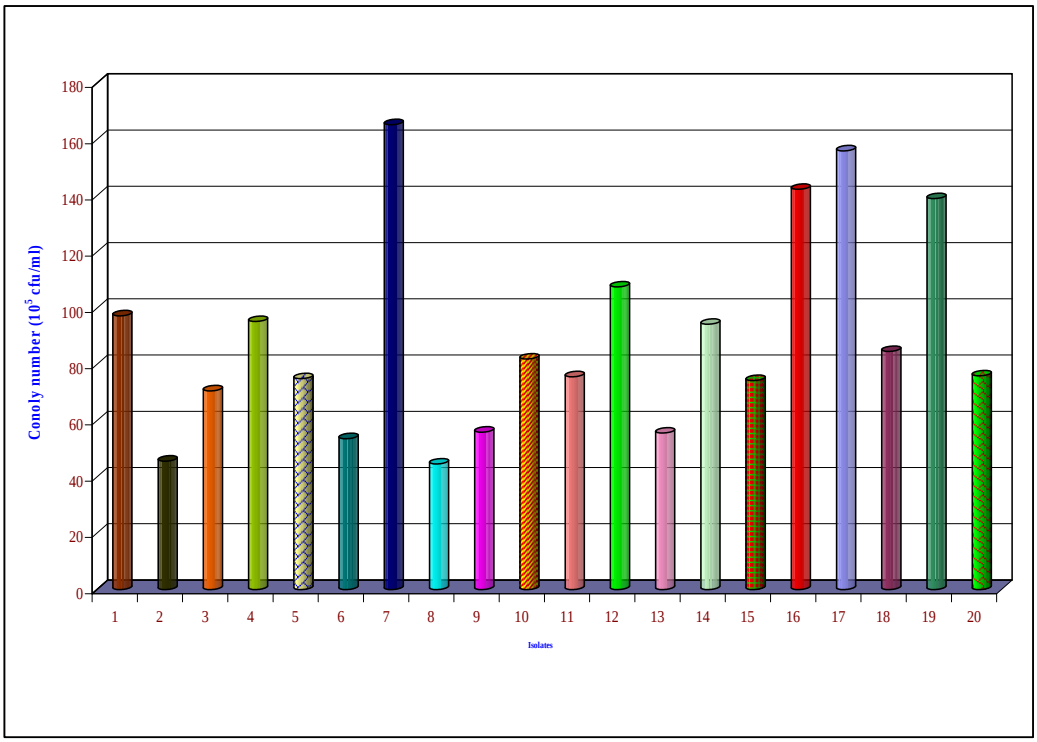


Fig. 4: Colonies of isolates of Xanthomonas axonopodis pv. punicae on MD-5 medium

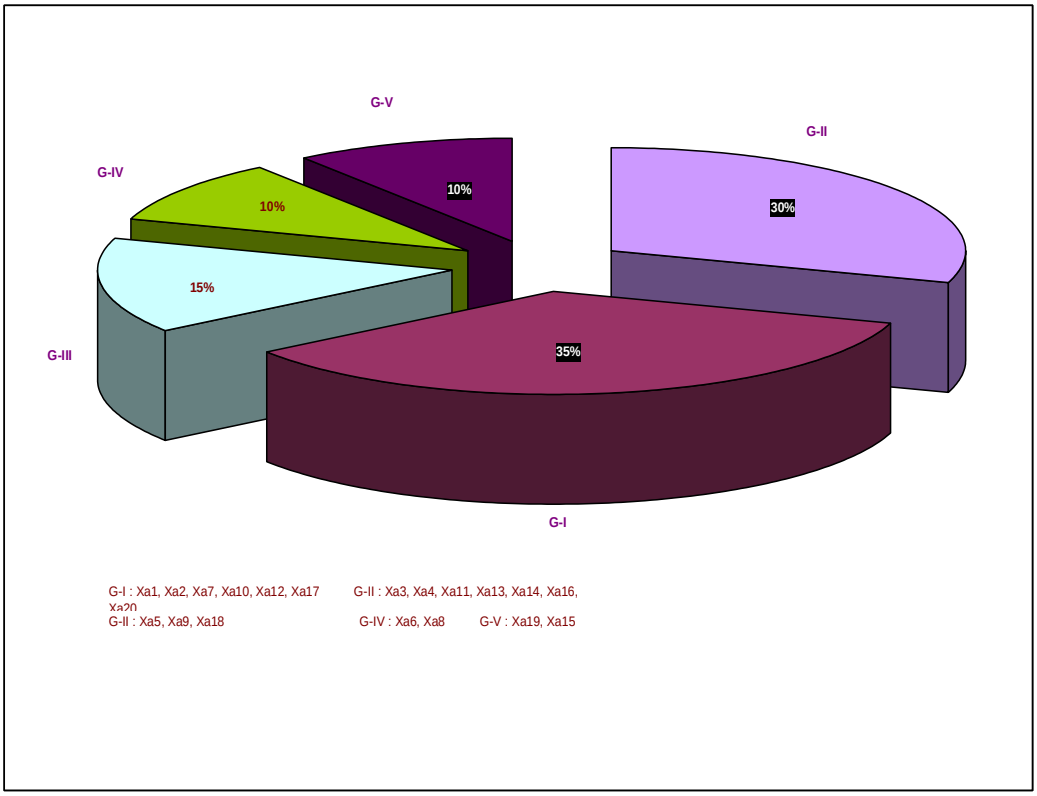


Fig. 5: Morphological groupings of twenty isolates of Xanthomonas axonopodis pv. punicae on MD-5 medium

Table 11: Banding profile of random primers for different isolates of *Xanthomonas axonopodis* pv. *punicae* causing bacterial blight in pomegranate

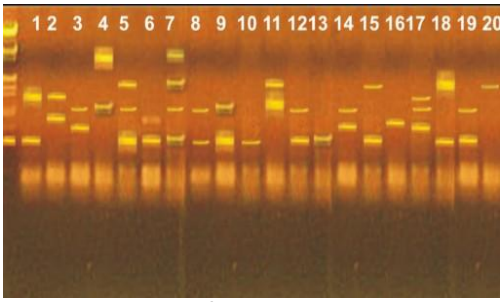
Sl. No.	Primers	Total bands	Polymorphic bands	Per cent polymorphism
1.	OPA06	5	4	80.00
2.	OPA09	6	5	83.33
3.	OPA12	7	5	71.43
4.	OPA19	5	4	80.00
5.	OPA20	3	3	100.00
6.	OPB02	7	6	85.71
7.	OPB03	5	5	100.00
8.	OPB04	2	1	50.00
9.	OPF07	5	5	100.00
10.	OPF09	8	6	75.00
11.	OPF10	7	7	100.00
	Total	60	51	

growth of the bacterium at these two temperature levels differed significantly and also was found superior to other temperature levels tested. However, the pathogen grew at a temperature levels ranged between 15^0C to 45^0C, but it failed to grow at the lowest and highest extreme temperature levels upto 10^0C and 50^0C, respectively (Fig. 7).

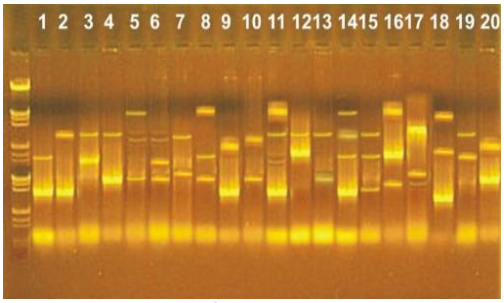
4.7.2 Effect of hydrogen ion concentration

This experiment was carried out to know the effect of pH on the growth of *X. axonopodis* pv. *punicae*. The effect on the growth was studied at varied pH levels ranged from 3.0 to 11.0 as detailed in “Material and Methods” and results obtained are presented in Table 14.

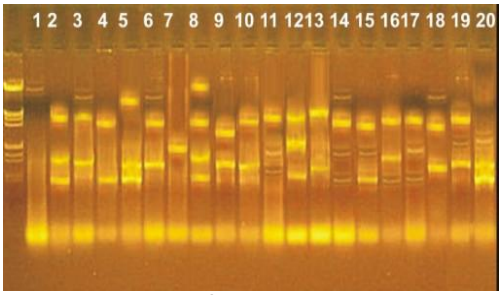
The data revealed that, number of bacterial colonies increased with increase in pH to certain range from 4.0 to 7.0 and thereafter colony numbers decreased with increase in pH indicating 7.0 and 7.5 were the optimum pH for maximum growth of the pathogen *X. axonopodis* pv. *punicae* (Fig. 8). The maximum number of bacterial colonies were recorded at pH 7.0 (145.33×10^5 cfu/ml) and were remained on par with pH 7.5. The next best pH levels, which significantly supported the good growth were 6.5 (81×10^5 cfu/ml), 8.0 (75.33×10^5 cfu/ml) and 8.5 (6.33×10^5 cfu/ml). Least growth of the pathogen was observed at lowest and highest pH levels of 4.0 (10×10^5 cfu/ml) and 10.5 (4×10^5 cfu/ml), respectively and no growth of the pathogen was recorded at pH levels of 3.0 and 11.0.



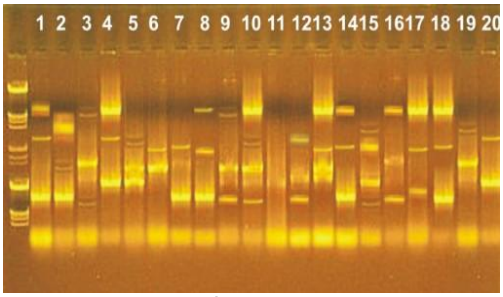
Primer OPA-19



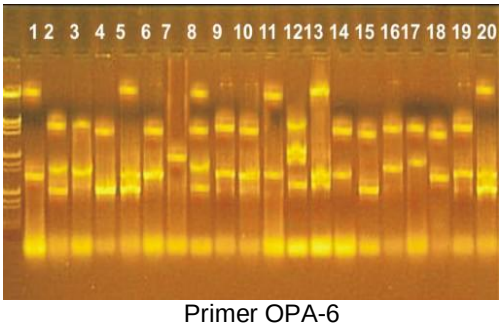
Primer OPA-12



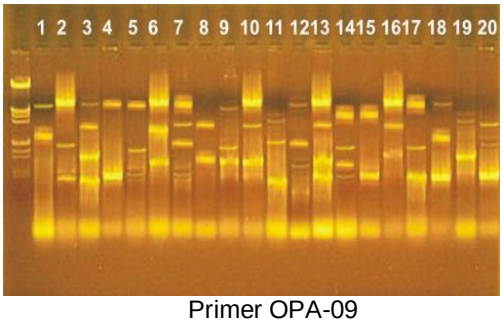
Primer OPB-03



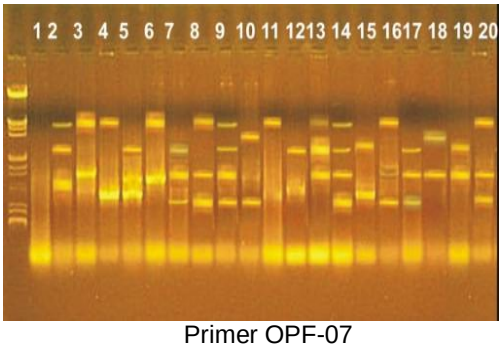
Primer OPF-10



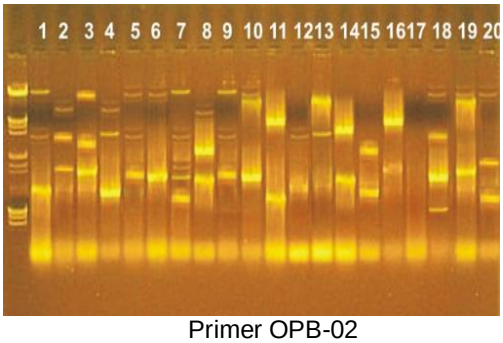
Primer OPA-6



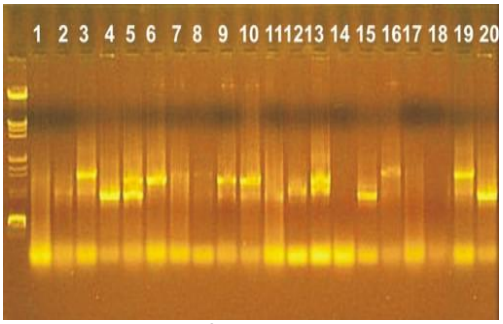
Primer OPA-09



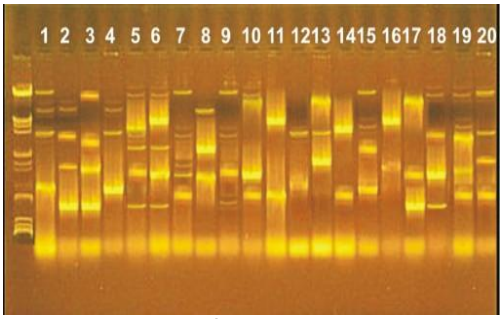
Primer OPF-07



Primer OPB-02



Primer OPA-20



Primer OPF-09

Plate 8: Banding pattern of 20 isolates of X. axonopodis pv.punicae by RAPD method

Table 12: Similarity coefficient of twenty isolates of *Xanthomonas axonopodis* pv. *punicae* based on RAPD analysis

Isolates	Xa_1	Xa_2	Xa_3	Xa_4	Xa_5	Xa_6	Xa_7	Xa_8	Xa_9	Xa_{10}	Xa_{11}	Xa_{12}	Xa_{13}	Xa_{14}	Xa_{15}	Xa_{16}	Xa_{17}	Xa_{18}	Xa_{19}	Xa_{20}
Xa_1	1.00																			
Xa_2	0.64	1.00																		
Xa_3	0.51	0.74	1.00																	
Xa_4	0.55	0.67	0.57	1.00																
Xa_5	0.56	0.67	0.61	0.55	1.00															
Xa_6	0.60	0.73	0.81	0.56	0.70	1.00														
Xa_7	0.62	0.68	0.69	0.53	0.57	0.68	1.00													
Xa_8	0.55	0.68	0.72	0.64	0.64	0.71	0.66	1.00												
Xa_9	0.66	0.74	0.75	0.58	0.61	0.77	0.75	0.69	1.00											
Xa_{10}	0.53	0.68	0.66	0.64	0.64	0.75	0.69	0.72	0.79	1.00										
Xa_{11}	0.64	0.73	0.61	0.63	0.63	0.70	0.61	0.58	0.68	0.57	1.00									
Xa_{12}	0.47	0.69	0.70	0.69	0.69	0.62	0.67	0.63	0.67	0.63	0.54	1.00								
Xa_{13}	0.65	0.68	0.75	0.57	0.71	0.71	0.55	0.62	0.69	0.65	0.57	0.63	1.00							
Xa_{14}	0.59	0.78	0.79	0.69	0.62	0.75	0.73	0.73	0.76	0.74	0.76	0.64	0.67	1.00						
Xa_{15}	0.47	0.59	0.70	0.58	0.61	0.59	0.59	0.57	0.63	0.63	0.58	0.71	0.63	0.61	1.00					
Xa_{16}	0.55	0.53	0.68	0.63	0.59	0.70	0.64	0.71	0.68	0.72	0.55	0.69	0.60	0.76	0.58	1.00				
Xa_{17}	0.58	0.58	0.59	0.56	0.57	0.61	0.67	0.57	0.74	0.58	0.53	0.68	0.59	0.67	0.60	0.61	1.00			
Xa_{18}	0.72	0.63	0.61	0.54	0.55	0.59	0.57	0.68	0.68	0.60	0.55	0.54	0.57	0.65	0.62	0.63	0.61	1.00		
Xa_{19}	0.54	0.59	0.74	0.53	0.54	0.65	0.70	0.59	0.80	0.71	0.58	0.64	0.59	0.61	0.64	0.57	0.59	0.53	1.00	
Xa_{20}	0.52	0.63	0.71	0.67	0.63	0.63	0.57	0.74	0.65	0.72	0.56	0.69	0.61	0.62	0.73	0.70	0.49	0.59	0.62	1.00

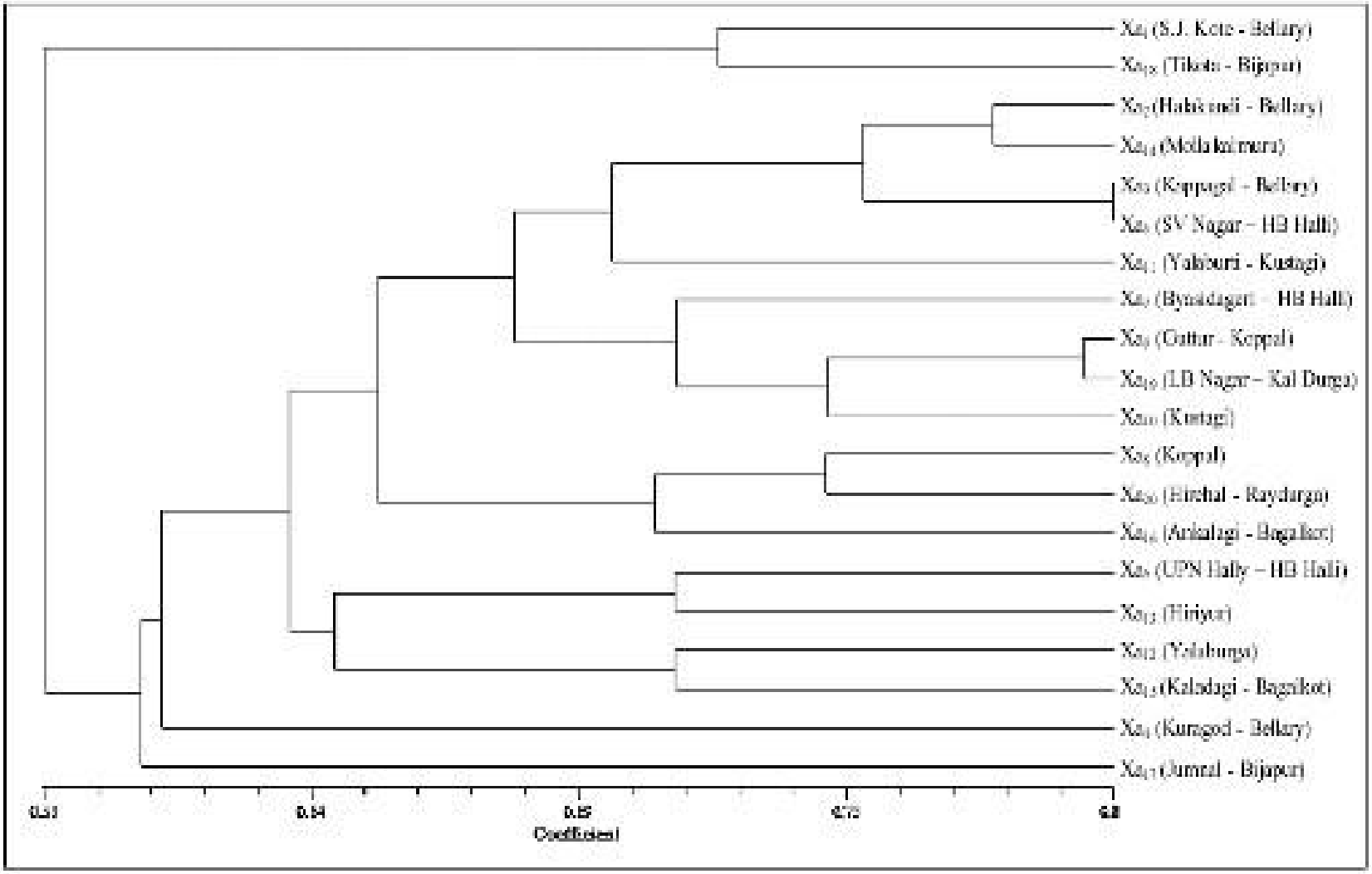


Fig.6: Dendrogram based on RAPD analysis of 20 isolates of Xanthomonas axonopodis pv. punicae causing bacterial blight of pomegranate

Table 13: Effect of temperature on the growth of *Xanthomonas axonopodis* pv. *punicae* under *in vitro* condition on MD-5 medium

Sl. No.	Temperature levels (^{0}C)	Number of colonies of the bacteria (10^5 cfu/ml)
1.	0	0.00 (1.00)*
2.	5	0.00 (1.00)
3.	10	0.00 (1.00)
4.	15	21.67 (4.75)
5.	20	41.00 (6.47)
6.	25	134.00 (11.62)
7.	30	147.33 (12.18)
8.	35	83.00 (9.16)
9.	40	36.00 (6.07)
10.	45	23.00 (4.90)
11.	50	0.00 (1.00)
	SEm±	0.17
	CD at 1%	0.69

* $\sqrt{x+1}$ transformed values

4.8 Disease epidemiology

4.8.1 Effect of dates of pruning and role of weather factors on bacterial blight incidence and severity

Field trials were conducted to study the effect of dates of pruning on bacterial blight intensity in relation to weather factors. The crop pruned during different months *viz.*, November 2006 (late Hastbahar), April 2007 (Ambiabahar), July 2007 (Mrigbahar) and September 2007 (Hastbahar) was constantly observed for the disease progress/decline. Correspondingly, weather factors prevailed during different seasons were also recorded and correlated with the disease.

Results indicated that, in late hastbahar season, when the crop was pruned during the month of November 2006, absolutely no disease incidence was recorded either on leaf or fruit till the end of 16th standard meteorological week of 2007 (Table 15). However, first symptom of the disease was noticed on fruit with initial incidence and severity of 12.34 per cent and 3.11 PDI, respectively during 17th week. The disease gradually progressed with age of the crop and reached the peak incidence (96.29 PDI) and severity (100 PDI) at the end of the cropping season during 28th week. Similarly, infection on leaf was noticed, bit late in twenty first week (2.96 PDI) and progressed further upto 32nd week (91.63 PDI).

Observations on weather parameters revealed that there was a continuous rainfall in all the weeks (ranged between 1.2 to 57 mm) right from 15th week till the harvest of the crop (32nd week). Maximum and minimum temperatures ranged between 30.1 to 40.4^{0}C and 22.4 to 27.3^{0}C, respectively and relative humidity of 68.6 to 86.0 per cent was prevailed during the period, which predisposed the plants for the attack of bacterial blight and for further progress (Fig. 9).

Table 14: Effect of pH on the growth of *Xanthomonas axonopodis* pv. *punicae* on MD-5 medium

Sl. No.	pH levels	Number of colonies of the bacterium (10^5 cfu/ml)
1.	3.0	0.00 (1.00)*
2.	4.0	10.00 (3.31)
3.	5.0	14.67 (3.95)
4.	5.5	21.00 (4.68)
5.	6.0	33.37 (5.87)
6.	6.5	81.00 (9.05)
7.	7.0	145.33 (12.09)
8.	7.5	136.67 (11.73)
9.	8.0	75.33 (8.73)
10.	8.5	63.33 (8.01)
11.	9.0	44.67 (6.75)
12.	9.5	29.00 (5.47)
13.	10.0	14.00 (3.86)
14.	10.5	4.00 (2.21)
15.	11.0	0.00 (1.00)
	SEm$\pm$ CD at 1%	0.21 0.82

* $\sqrt{\times+1}$ transformed values

Observations made during ambiabahar (April 2007) cropping season indicated that, the crop was completely free from infection till 23rd week of 2007. Initial infection of the disease both on leaf (0.62 PDI) and fruit (15.73% incidence and 2.57 PDI) was recorded during twenty fourth week. Thereafter, disease gradually increased for the further period and reached the highest severity on leaf (39.21 PDI) and fruit (incidence of 67.06% and severity of 45.13 PDI) by 37th and 38th week, respectively. Further, disease severity reduced gradually to the lowest severity of 4.21 PDI on leaf and 8.24 PDI on fruit by 42nd and 44th week of 2007, respectively (Fig. 10).

The weather factors remained same as that of previous season (late hastbahar) till the disease reached its peak severity, but there was a decrease in maximum (35.6 to 28.9)

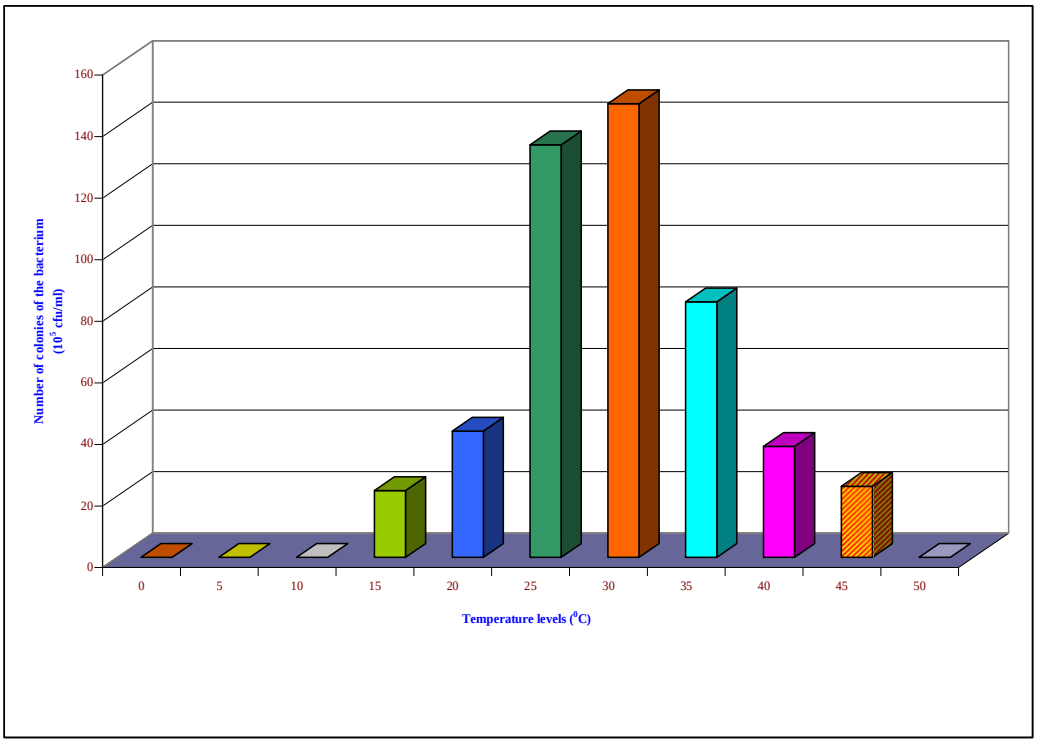


Fig. 7: Effect of different temperature levels on the growth of Xanthomonas axonopodis pv. punicae on MD-5 medium

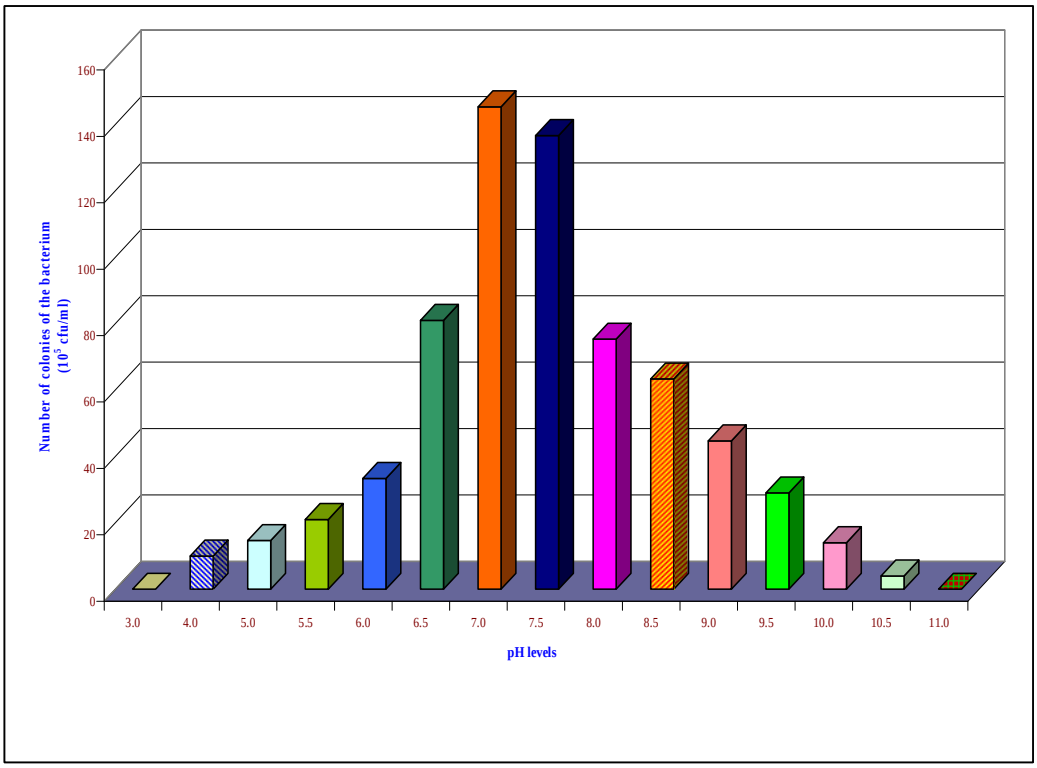


Fig. 8: Growth of Xanthomonas axonopodis pv. punicae at different pH levels on MD-5 medium

Table 15: Effect of dates of pruning on bacterial blight development in pomegranate during late Hastbahar (November-2006 pruned) and Ambiabahar (April-2007 pruned) in relation to weather factors

Standard meteorological weeks	Mean temperature (^{0}C)		Mean relative humidity (%)	Total rainfall (mm)	November, 2006 (late hastbahar)			April, 2007 (ambiabahar)		
					Leaf	Fruit		Leaf	Fruit	
	Maximum	Minimum			Per cent disease index	Per cent incidence	Per cent disease index	Per cent disease index	Per cent incidence	Per cent disease index
45th	27.4	23.0	86.0	42.4	- *	-	-	-	-	-
46th	27.4	22.4	90.3	0.0	-	-	-	-	-	-
47th	28.0	21.0	90.7	1.6	-	-	-	-	-	-
48th	29.3	20.4	87.8	0.0	-	-	-	-	-	-
49th	27.9	17.4	88.4	0.0	0.00	-	-	-	-	-
50th	28.9	18.5	85.8	0.0	0.00	-	-	-	-	-
51st	26.9	15.6	93.8	0.0	0.00	-	-	-	-	-
52nd	26.9	15.7	93.1	0.0	0.00	-	-	-	-	-
1st	27.4	15.4	92.6	0.0	0.00	-	-	-	-	-
2nd	28.1	16.9	90.8	0.0	0.00	-	-	-	-	-
3rd	29.8	17.4	91.0	0.0	0.00	0.00	0.00	-	-	-
4th	33.1	18.9	78.6	0.0	0.00	0.00	0.00	-	-	-
5th	34.3	15.1	69.7	0.0	0.00	0.00	0.00	-	-	-
6th	34.3	17.9	73.3	0.0	0.00	0.00	0.00	-	-	-
7th	30.4	14.8	76.6	0.0	0.00	0.00	0.00	-	-	-
8th	35.5	17.0	72.3	0.0	0.00	0.00	0.00	-	-	-
9th	36.6	20.1	80.0	0.0	0.00	0.00	0.00	-	-	-
10th	37.6	21.7	74.5	0.0	0.00	0.00	0.00	-	-	-
11th	39.0	21.1	76.0	0.0	0.00	0.00	0.00	-	-	-
12th	40.5	25.5	71.3	0.0	0.00	0.00	0.00	-	-	-
13th	40.7	27.7	74.0	0.0	0.00	0.00	0.00	-	-	-
14th	39.3	27.3	75.6	15.6	0.00	0.00	0.00	-	-	-
15th	37.6	24.1	76.1	4.2	0.00	0.00	0.00	-	-	-
16th	41.2	29.4	69.6	1.2	0.00	0.00	0.00	-	-	-

Contd….

Standard meteorological weeks	Mean temperature (^{0}C)		Mean relative humidity (%)	Total rainfall (mm)	November, 2006 (late hastbahar)			April, 2007 (ambiabahar)		
					Leaf	Fruit		Leaf	Fruit	
	Maximum	Minimum			Per cent disease index	Per cent incidence	Per cent disease index	Per cent disease index	Per cent incidence	Per cent disease index
17th	40.2	27.3	80.0	1.2	0.00	12.34	3.11	0.00	-	-
18th	40.1	26.7	79.1	0.0	0.00	15.29	7.76	0.00	-	-
19th	40.4	26.4	77.7	5.6	0.00	22.42	17.38	0.00	-	-
20th	37.9	27.0	84.4	28.8	0.00	24.61	27.49	0.00	-	-
21st	35.8	24.4	83.7	12.8	2.96	32.61	41.52	0.00	0.00	0.00
22nd	34.6	24.6	83.4	17.4	5.80	35.43	54.44	0.00	0.00	0.00
23rd	33.8	23.1	79.3	7.6	16.79	42.68	64.76	0.00	0.00	0.00
24th	30.1	24.3	84.6	57.0	28.32	48.73	69.31	0.62	15.73	2.57
25th	33.6	24.4	74.3	1.2	35.68	56.73	81.20	1.11	22.6	5.71
26th	34.6	24.9	68.6	12.3	53.82	65.82	90.99	2.09	30.79	8.61
27th	34.2	22.9	81.7	4.80	68.36	81.73	96.10	5.06	35.77	11.84
28th	33.8	22.6	86.0	16.4	72.49	96.29	100.00	7.81	35.86	12.72
29th	32.1	22.8	77.9	Tr	83.64	-- **	--	8.25	35.92	14.14
30th	33.8	22.6	71.1	5.2	84.36	--	--	15.68	34.84	18.99
31st	32.9	22.4	71.8	1.2	88.19	--	--	24.44	32.14	17.12
32nd	33.0	23.3	77.1	2.8	91.63	--	--	26.78	33.28	18.64
33rd	32.9	22.8	72.9	0.0	--	--	--	27.33	35.17	16.84
34th	33.2	22.6	80.4	24.0	--	--	--	29.00	39.72	19.92
35th	29.4	21.2	82.3	91.8	--	--	--	32.47	42.62	22.39
36th	32.2	21.4	76.6	54.0	--	--	--	34.28	49.28	28.45
37th	32.7	21.8	78.1	0.0	--	--	--	39.61	64.19	41.40
38th	35.0	19.5	70.9	27.4	--	--	--	20.98	67.06	45.13
39th	35.6	21.2	75.0	0.0	--	--	--	15.74	63.92	41.33
40th	27.7	19.1	87.1	12.0	--	--	--	13.28	53.41	33.37
41st	30.7	20.9	84.1	8.2	--	--	--	9.65	47.08	27.92
42nd	31.3	19.7	71.1	4.5	--	--	--	4.21	33.67	16.43
43rd	29.7	12.1	63.6	0.0	--	--	--	0.00	30.46	13.49
44th	30.3	12.3	54.8	0.0	--	--	--	0.00	19.55	8.24
45th	28.9	14.1	71.3	0.0	--	--	--	0.00	0.00	0.00
46th	29.5	14.1	84.1	0.0	--	--	--	0.00	0.00	0.00

* : Foliage and fruit yet to be develop

** : End of the cropping season

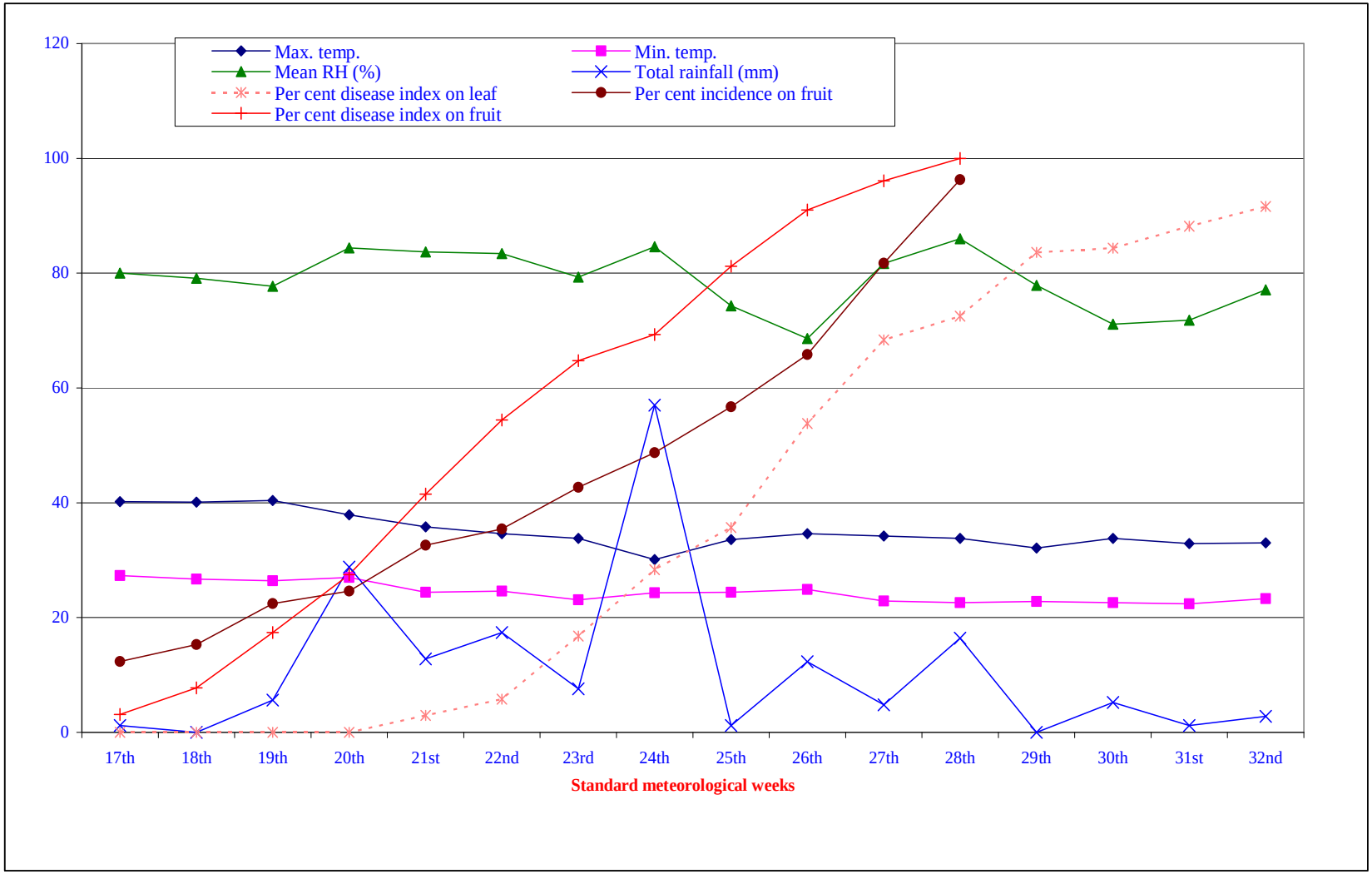


Fig. 9: Development and spread of bacterial blight in Late Hastbahar (November-2006 pruned) in relation to weather factors

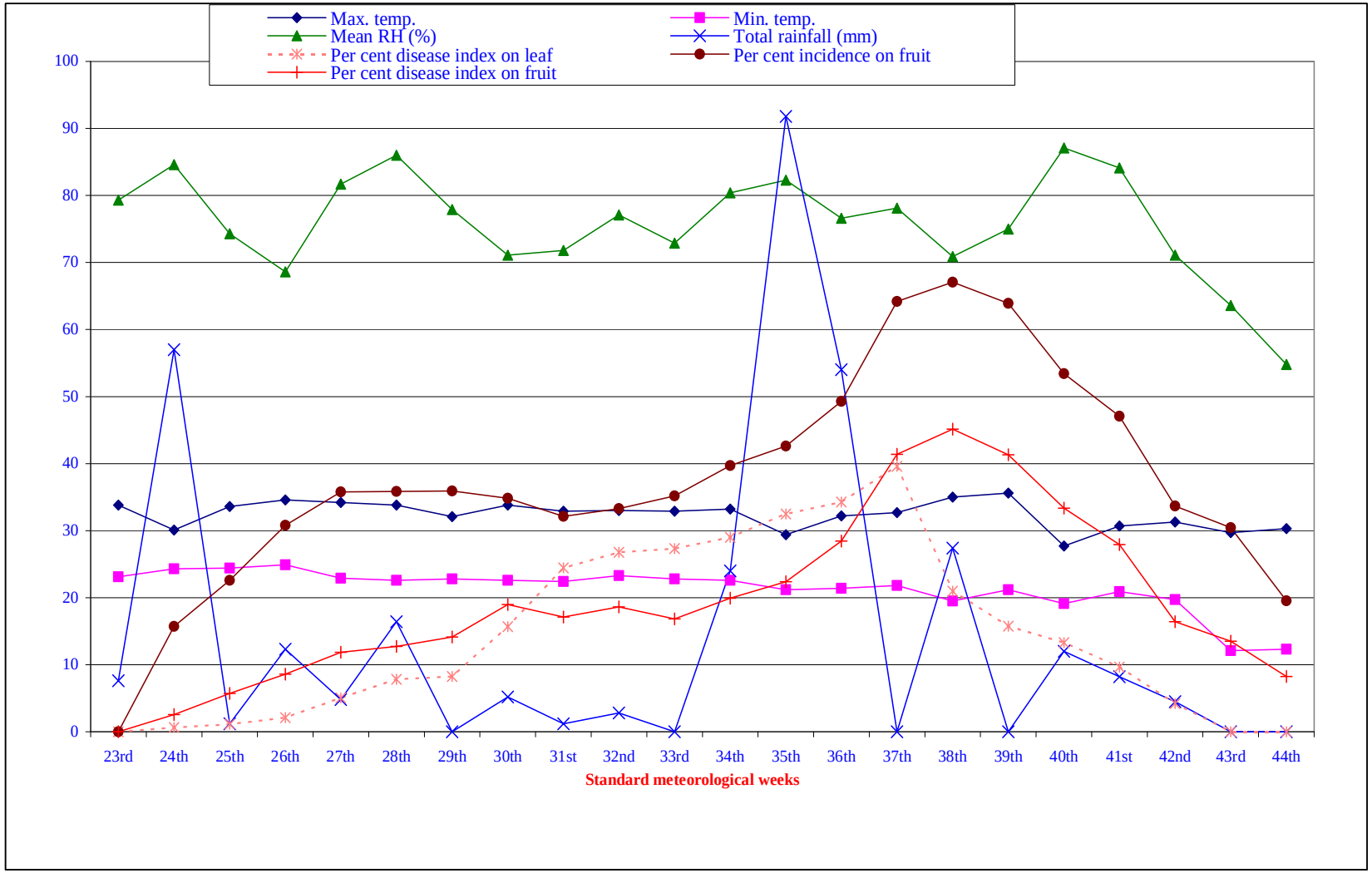


Fig. 10: Development and spread of bacterial blight in Ambiabahar (April-2007 pruned) in relation to weather factors

Disease progress during Mrigbahar

Disease suppression during Hasthbahar

Plate 9: Indication of disease progress during Mrigbahar and disease suppression during Hastbahar owing to weather factors

Table 16: Effect of dates of pruning on bacterial blight development in pomegranate during Mrigbahar (July-2007 pruned) and Hastbahar (September-2007 pruned) in relation to weather factors

Standard meteorological weeks	Mean temperature (^{0}C)		Mean relative humidity (%)	Total rainfall (mm)	July, 2007 (Mrigbahar)			September, 2007 (Hastbahar)		
					Leaf	Fruit		Leaf	Fruit	
	Maximum	Minimum			Per cent disease index	Per cent incidence	Per cent disease index	Per cent disease index	Per cent incidence	Per cent disease index
29th	33.6	24.4	74.3	1.2	- *	-	-	-	-	-
30th	34.6	24.9	68.6	12.3	-	-	-	-	-	-
31th	34.2	22.9	81.7	4.8	-	-	-	-	-	-
32th	33.8	22.6	86.0	16.4	-	-	-	-	-	-
33st	32.1	22.8	77.9	0.0	-	-	-	-	-	-
34nd	33.8	22.6	71.1	5.23	-	-	-	-	-	-
35rd	32.9	22.4	71.8	1.2	0.00	-	-	-	-	-
36th	33.0	23.3	77.1	2.8	0.00	-	-	-	-	-
37th	32.9	22.8	72.9	0.00	0.00	-	-	-	-	-
38th	33.2	22.6	80.4	24.2	0.00	0.00	0.00	-	-	-
39th	29.4	21.2	82.3	91.8	0.00	0.00	0.00	-	-	-
40th	32.2	21.4	76.6	54.0	1.60	4.72	3.38	-	-	-
41th	32.7	21.8	78.1	0.0	7.04	6.40	5.06	-	-	-
42st	35.0	19.5	70.9	27.4	14.94	9.25	7.51	0.00	-	-
43nd	35.6	21.2	75.0	0.00	12.83	8.89	14.52	3.26	-	-
44rd	27.7	19.1	87.1	12.0	8.27	14.91	10.80	5.82	-	-
45th	30.7	20.9	84.1	8.2	20.36	42.36	16.06	8.54	9.45	3.68
46th	31.3	19.7	71.1	4.5	38.39	64.07	36.50	10.63	15.63	7.42
47th	29.7	12.1	63.6	0.0	40.27	66.31	40.04	11.41	18.32	10.31
48th	30.3	12.3	54.8	0.0	43.48	67.78	44.33	10.21	14.41	12.63

Standard meteorological weeks	Mean temperature (^{0}C)		Mean relative humidity (%)	Total rainfall (mm)	July, 2007 (Mrigbahar)			September, 2007 (Hastbahar)		
					Leaf	Fruit		Leaf	Fruit	
	Maximum	Minimum			Per cent disease index	Per cent incidence	Per cent disease index	Per cent disease index	Per cent incidence	Per cent disease index
49th	28.9	14.1	71.3	0.0	37.40	65.84	43.04	9.81	10.36	8.74
50th	29.5	14.1	84.1	0.0	32.61	63.82	38.29	7.56	8.76	6.31
51st	28.8	15.2	84.3	0.0	33.72	61.39	31.43	4.33	5.21	2.82
52nd	32.7	14.3	77.5	0.0	28.51	22.08	14.22	3.72	2.38	1.52
1st	30.3	11.3	83.3	0.0	26.38	19.08	10.80	0.00	0.00	0.00
2nd	31.6	10.2	75.0	0.0	25.06	13.80	6.97	0.00	0.00	0.00
3rd	32.7	11.5	67.7	0.0	15.28	9.55	3.81	0.00	0.00	0.00
4th	32.2	15.2	70.3	0.0	11.43	6.93	2.72	0.00	0.00	0.00
5th	31.3	18.6	83.0	0.0	9.62	4.20	2.58	0.00	0.00	0.00
6th	32.3	17.9	75.3	0.0	8.52	2.79	1.39	0.00	0.00	0.00
7th	34.9	21.3	72.4	0.0	5.74	2.22	0.24	0.00	0.00	0.00
8th	35.5	16.3	72.1	0.0	5.18	1.48	0.18	0.00	0.00	0.00
9th	36.1	15.0	68.3	0.0	0.00	0.00	0.00	0.00	0.00	0.00
10th	35.3	16.9	73.6	0.0	-- **	--	--	0.00	0.00	0.00
11th	34.5	20.2	72.9	9.2	--	--	--	0.00	0.00	0.00
12th	33.4	20.4	85.9	46.8	--	--	--	0.00	0.00	0.00
13th	35.6	20.3	77.6	0.0	--	--	--	4.53	9.31	3.92
14th	35.4	22.7	69.1	0.0	--	--	--	0.00	0.00	0.00
15th	36.9	20.8	76.0	0.0	--	--	--	0.00	0.00	0.00

* : Foliage and fruit yet to be develop ** : End of the cropping season

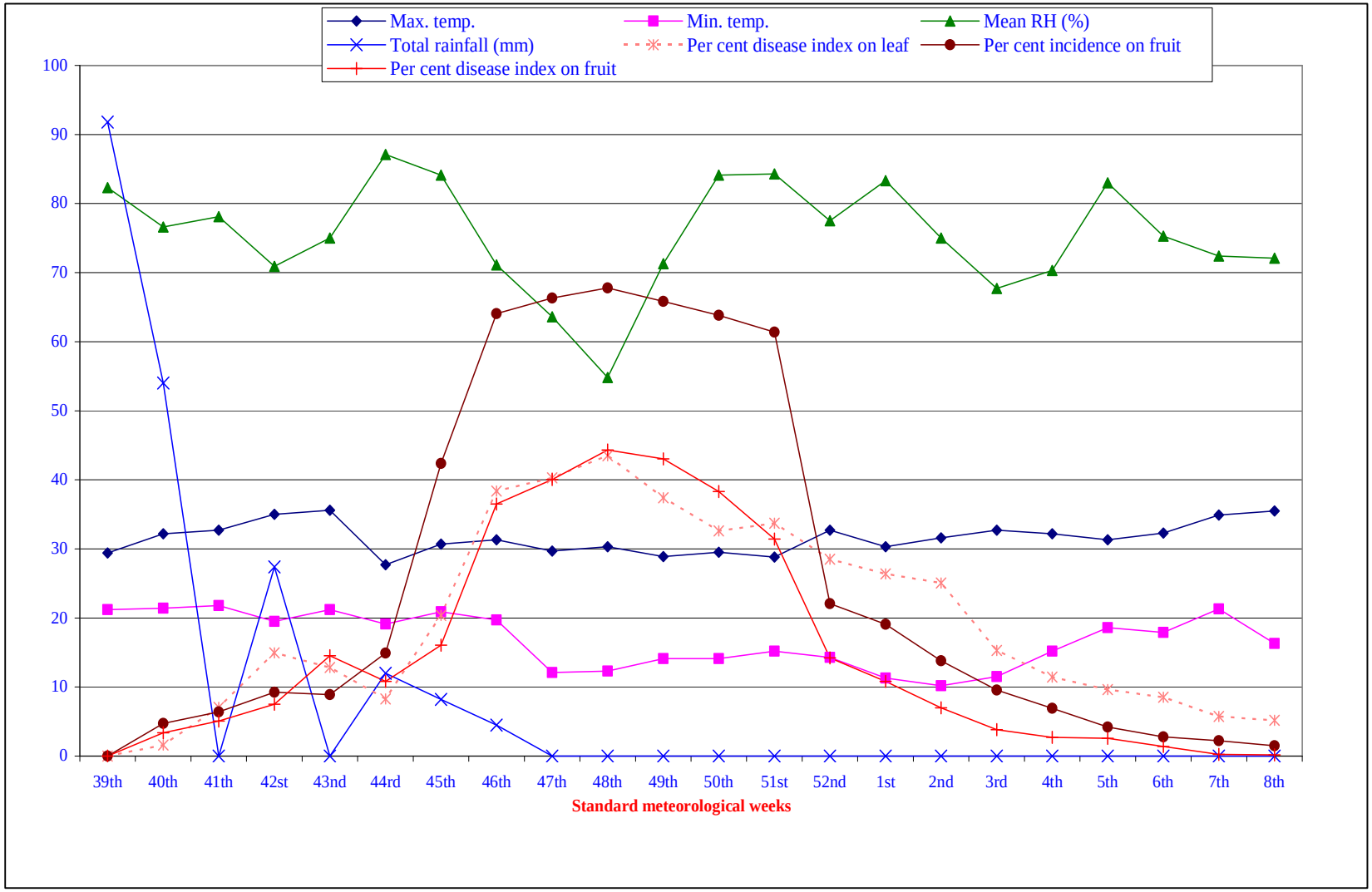


Fig. 11: Development and spread of bacterial blight in Mrigbahar (July-2007 pruned) in relation to weather factors

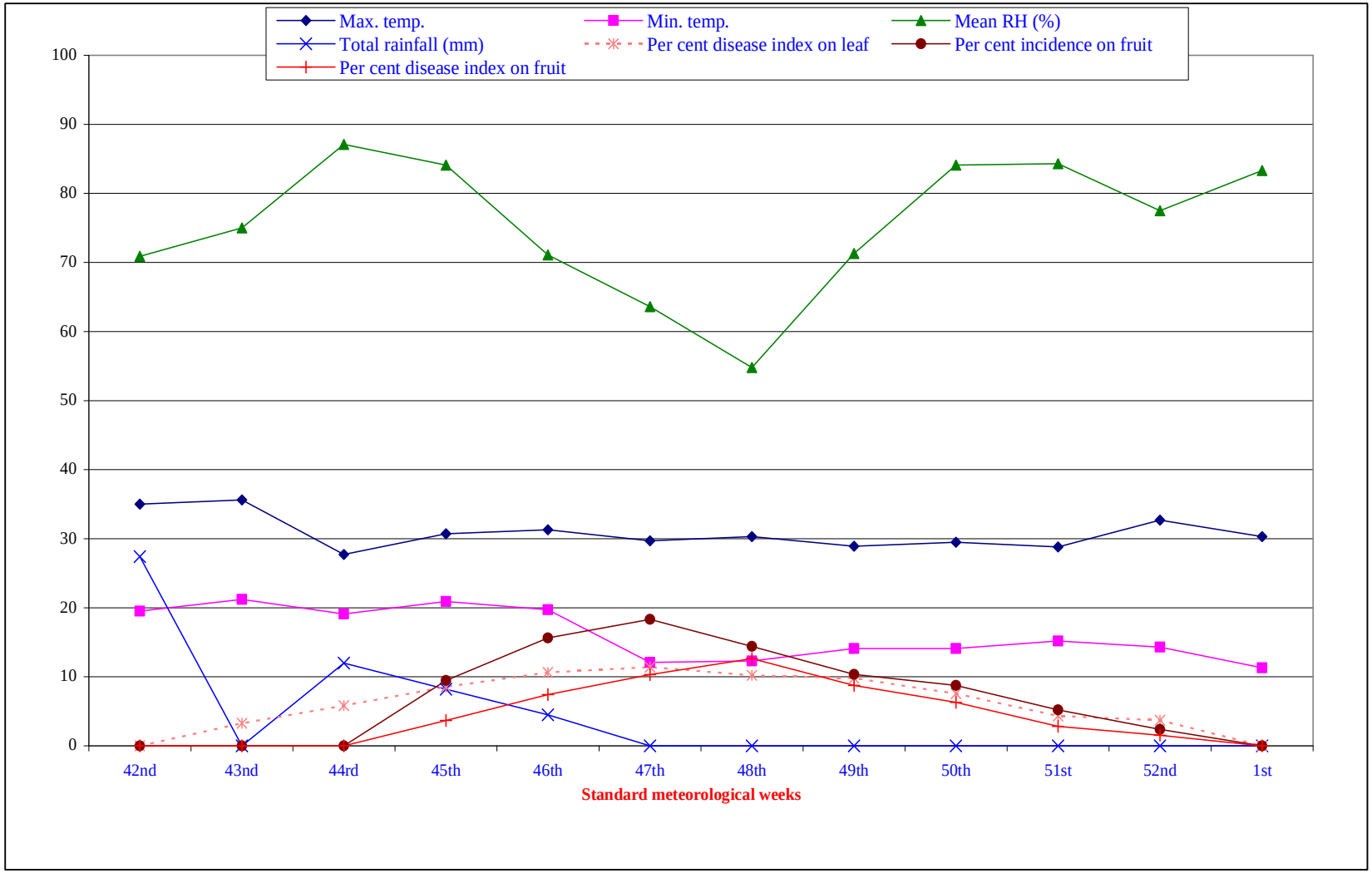


Fig. 12: Development and spread of bacterial blight in Hastbahar (September-2007 pruned) in relation to weather factors

Table 17: Correlation coefficients between disease and weather variables

Cropping seasons	Correlation coefficient 'r'			
	Max. temp. (^{0}C)	Min. temp. (^{0}C)	Relative humidity (%)	Rainfall (mm)
November 2006 (Late Hastbahar)				
PDI on leaf	-0.473*	-0.743**	-0.509**	-0.491**
Per cent incidence on fruit	-0.740**	-0.859**	0.229NS	0.187 NS
PDI on fruit	-0.859**	-0.878**	0.135 NS	0.257 NS
April 2007 (Ambiabahar)				
PDI on leaf	-0.035 NS	-0.276 NS	-0.054 NS	0.244 NS
Per cent incidence on fruit	0.223 NS	-0.006 NS	0.262 NS	0.039 NS
PDI on fruit	0.144 NS	-0.096 NS	0.176 NS	0.029 NS
July 2007 (Mrigbahar)				
PDI on leaf	-0.443**	-0.622**	-0.349**	-0.449**
Per cent incidence on fruit	-0.572**	-0.388**	-0.238**	-0.298**
PDI on fruit	-0.520**	-0.379**	-0.307**	-0.295**
September 2007 (Hastbahar)				
PDI on leaf	-0.590**	-0.415**	-0.381**	-0.535**
Per cent incidence on fruit	-0.201 NS	-0.075 NS	-0.697**	0.117 NS
PDI on fruit	-0.333**	-0.451**	-0.864**	-0.254**

** : Significant at 1% level
* : Significant at 5% level
NS : Non-significant

and minimum (21.2 to 12.1^{0}C) temperature from 38th week to 44th week, wherein the disease gradually reduced to the lowest severity.

In mrigbahar cropping season, when the crop was pruned during July 2007, initial symptoms of the disease (both on leaf and fruit) were noticed at the end of 40th week (Table 16). The gradual increase in incidence and severity of the disease was observed till 48th week with peak severity of 43.48 PDI on leaf, while on fruit, it was still maximum with incidence and severity of 67.78 per cent and 44.33 PDI, respectively. From 49th week onwards disease gradually decreased to the lowest amount by 8th week of 2008 before the harvest of the crop (Plate 9).

The data on weather variables indicated that, there was an intermittent rainfall from the day of disease onset (40th week of 2007) till its peak severity (48th week of 2007) with almost similar temperature and relative humidity levels prevailed as in case of earlier seasons. But from 49th week of 2007 onwards till the end of 8th week of 2008, absolutely there

was no rainfall and minimum temperature came down and prevailed in the range of 10.2 to 15.2^{0}C for the longer period of two months (Fig. 11).

In hastbahar cropping season (September 2007), the disease symptoms were noticed in the beginning of the cropping period and rate of spread of the disease was also very slow and only for the shorter period as compared to the rest of the seasons. The disease was first observed on leaf with 3.26 PDI during 43rd week (2007) and slowly progressed for a shorter period till 47th week amounting 11.41 PDI as highest severity and gradually declined to 3.72 PDI by the end of 52nd week of 2007. Similar trend was noticed on fruits that, disease started with initial infection of 9.45 per cent and severity of 3.68 PDI during 45th week and slowly progressed for a shorter period of two weeks amounting to the highest incidence and severity of 18.32 per cent and 12.63 PDI, respectively. Thereafter, disease was gradually reduced in subsequent weeks and the crop was found completely free of infection from 1st week of 2008 till harvest (Plate 9).

The weather data revealed that there was an intermittent rainfall from 38th week to 46th week (4.5 – 91.8 mm) with maximum and minimum temperature ranged between 35.6 to 27.7^{0}C and relative humidity between 87.1 to 63.6 per cent prevailed during the season favoured the disease progress for a shorter period from 41st to 48th week. The weather conditions prevailed from 49th week of 2007 to 12th week of 2008 were found uncongenial for disease development and spread (Fig. 12).

4.8.1.1 Correlation with weather factors

Weather factors such as temperature, relative humidity and rainfall played a major role in bacterial blight development and spread. Correlation between the disease development and weather factors was well established and briefed as under.

4.8.1.2 Role of maximum temperature

Generally, the maximum temperature had negative influence on the disease progress/decline as observed in many cases during different seasons (Table 17). In late hastbahar cropping season, correlation between the disease progress either on leaf or fruit and maximum temperature followed the significant negative relationship. The correlation followed the similar trend in mrigbahar (July, 2007) and hastbahar (September 2007) cropping season, which revealed that influence of maximum temperature was significantly negative with disease incidence and severity observed both on leaf and fruit. On the contrary to this, during ambiabahar cropping season (April 2007), maximum temperature had a positive non-significant effect on the disease incidence ($r = 0.223$) and severity ($r = 0.144$) of fruits. However, the effect was negative ($r = -0.035$) in respect of leaf severity.

4.8.1.3 Influence of minimum temperature

The minimum temperature prevailed during late hastbahar, hastbahar and mrigbahar seasons on all the disease components had a significant negative influence except that, per cent incidence on fruit in hastbahar cropping season followed the non-significant negative relationship with minimum temperature ($r = -0.075$). In ambiabahar cropping season, minimum temperature had negative non-significant relationship with the disease.

4.8.1.4 Effect of relative humidity

The effect of RH was significantly positive with disease incidence on fruits as observed during late hastbahar and ambiabahar cropping season. However, the level of significance was at only 1 per cent indicating narrow fluctuation in relative humidity does not had much impact on the disease progress/decline. The effect of relative humidity was found significantly negative on the incidence and severity of the disease recorded during rest of the seasons (mrigbahar and hastbahar).

4.8.1.5 Effect of rainfall

Rainfall is an important weather factor, found to play a major role on bacterial blight development and spread. Rainfall had both positive and negative influence on the disease development during different seasons.

Results of late hastbahar season revealed that continuous rainfall from first week of May till the end of July had a non-significant positive influence on the incidence and severity

Table 18a: Observed and estimated bacterial blight incidence and severity during late hastbahar cropping season (November, 2006)

Standard meteorological weeks	Per cent disease index on leaf			Per cent incidence on fruit			Per cent disease index on fruit		
	Observed	Predicted	Difference	Observed	Predicted	Difference	Observed	Predicted	Difference
16th	-	-	-	1.00	1.59	-0.59	1.00	1.32	-0.32
17th	-	-	-	3.65	3.30	0.36	2.03	2.63	-0.60
18th	-	-	-	4.04	4.20	-0.16	2.96	3.56	-0.60
19th	-	-	-	4.84	5.09	-0.24	4.29	4.37	-0.09
20st	1.00	0.32	0.68	5.06	3.98	1.09	5.34	3.65	1.68
21rd	1.99	4.03	2.04	5.80	6.72	-0.92	6.52	6.99	-0.48
22nd	2.60	4.20	-1.59	6.04	6.42	0.39	7.45	7.10	0.34
23rd	4.22	7.25	-3.04	6.61	8.47	-1.86	8.02	9.56	-1.45
24th	5.42	5.65	-0.23	7.05	7.53	-0.48	8.39	9.63	-1.00
25th	6.06	6.59	-0.53	7.60	6.82	0.78	9.07	8.38	0.69
26th	7.40	6.57	0.83	8.17	7.40	0.77	9.59	8.91	0.68
27th	8.33	6.95	1.37	9.09	8.45	0.63	9.85	9.28	0.57
28th	8.57	6.62	1.96	9.86	8.84	1.02	10.05	9.48	0.57
29th	9.20	8.53	0.67	-	-	-	-	-	-
30th	9.24	9.44	-0.19	-	-	-	-	-	-
31st	9.44	9.90	-0.46	-	-	-	-	-	-
32nd	9.62	7.70	1.22	-	-	-	-	-	-

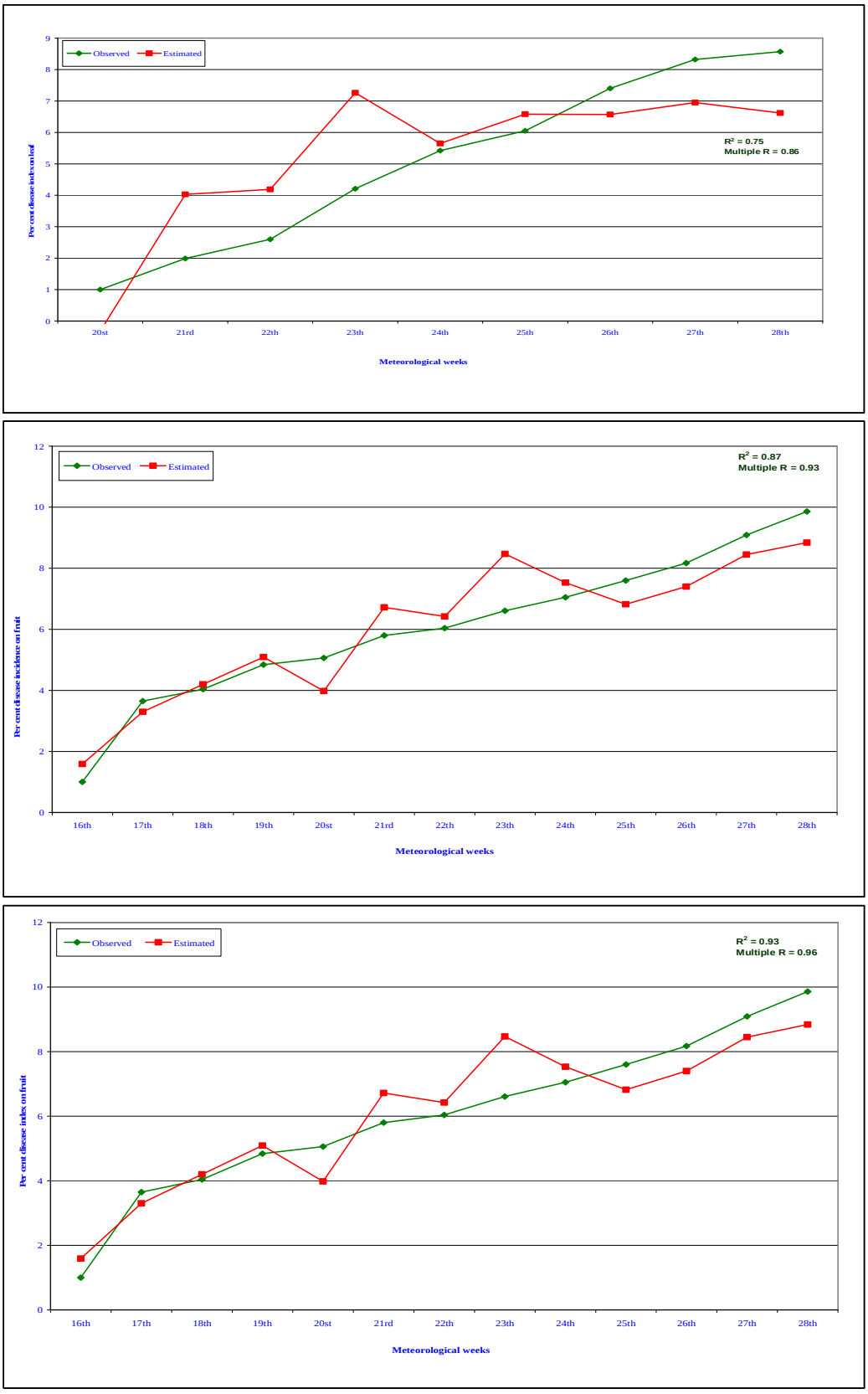
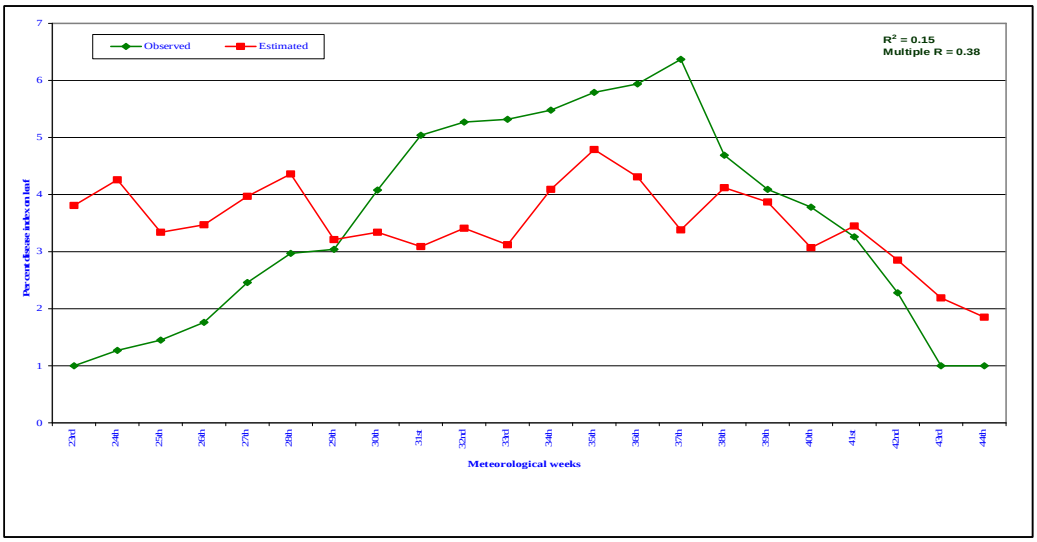
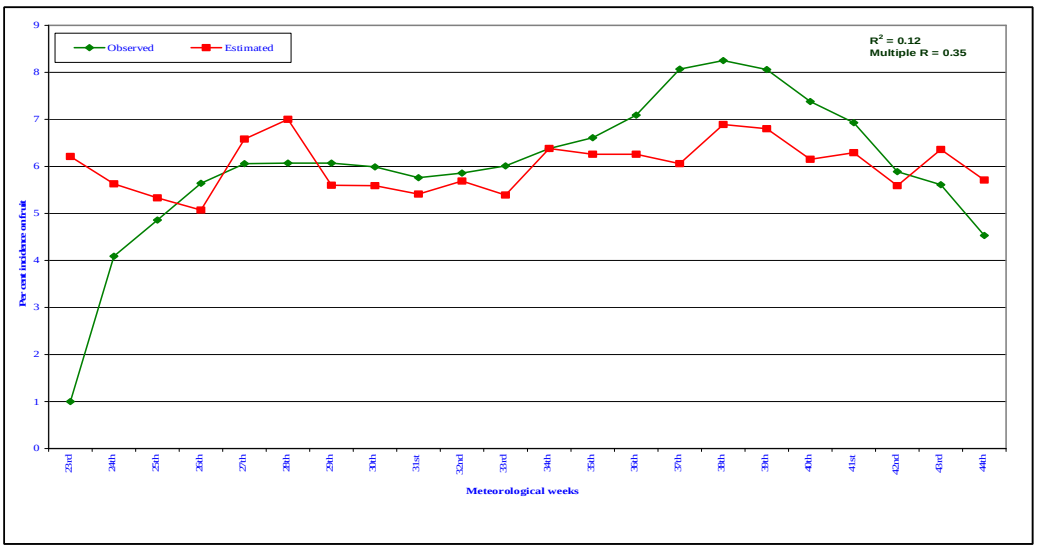


Fig.13: Observed and estimated bacterial blight incidence and severity during late Hastbahar cropping season (November-2006 pruned)

Table 18b: Observed and estimated bacterial blight incidence and severity during ambiabahar cropping season (April, 2007)

Standard meteorological weeks	Per cent disease index on leaf			Per cent incidence on fruit			Per cent disease index on fruit		
	Observed	Predicted	Difference	Observed	Predicted	Difference	Observed	Predicted	Difference
23rd	1.00	3.81	-2.81	1.00	6.21	-5.21	1.00	4.47	-3.47
24th	1.27	4.26	-2.98	4.09	5.63	-1.54	1.89	3.68	-1.79
25th	1.45	3.34	-1.88	4.86	5.33	-0.47	2.59	3.46	-0.87
26th	1.76	3.47	-1.71	5.64	5.07	0.57	3.10	3.13	-0.03
27th	2.46	3.97	-1.51	6.06	6.58	-0.51	3.58	4.89	-1.31
28th	2.97	4.36	-1.39	6.07	7.00	-0.92	3.70	5.35	-1.64
29th	3.04	3.21	-0.17	6.07	5.60	0.47	3.89	3.82	0.07
30th	4.08	3.34	0.75	5.99	5.59	0.39	4.47	3.79	0.68
31st	5.04	3.09	1.94	5.76	5.41	0.35	4.25	3.60	0.65
32nd	5.27	3.41	1.86	5.86	5.69	0.16	4.43	3.89	0.54
33rd	5.32	3.12	2.20	6.01	5.39	0.62	4.22	3.58	0.64
34th	5.48	4.09	1.39	6.38	6.38	0.00	4.57	4.63	-0.06
35th	5.79	4.79	1.00	6.61	6.26	0.35	4.84	4.39	0.44
36th	5.94	4.31	1.63	7.09	6.26	0.83	5.43	4.47	0.96
37th	6.37	3.38	2.99	8.07	6.06	2.02	6.51	4.35	2.16
38th	4.69	4.12	0.56	8.25	6.89	1.36	6.79	5.27	1.53
39th	4.09	3.87	0.22	8.06	6.80	1.26	6.51	5.19	1.32
40th	3.78	3.07	0.71	7.38	6.15	1.23	5.86	4.50	1.36
41st	3.26	3.45	-0.19	6.93	6.29	0.65	5.38	4.61	0.76
42nd	2.28	2.85	-0.56	5.89	5.59	0.30	4.18	3.86	0.31
43rd	1.00	2.19	-1.19	5.61	6.36	-0.75	3.81	4.92	-1.12
44th	1.00	1.85	-0.85	4.53	5.71	-1.17	3.04	4.18	-1.14





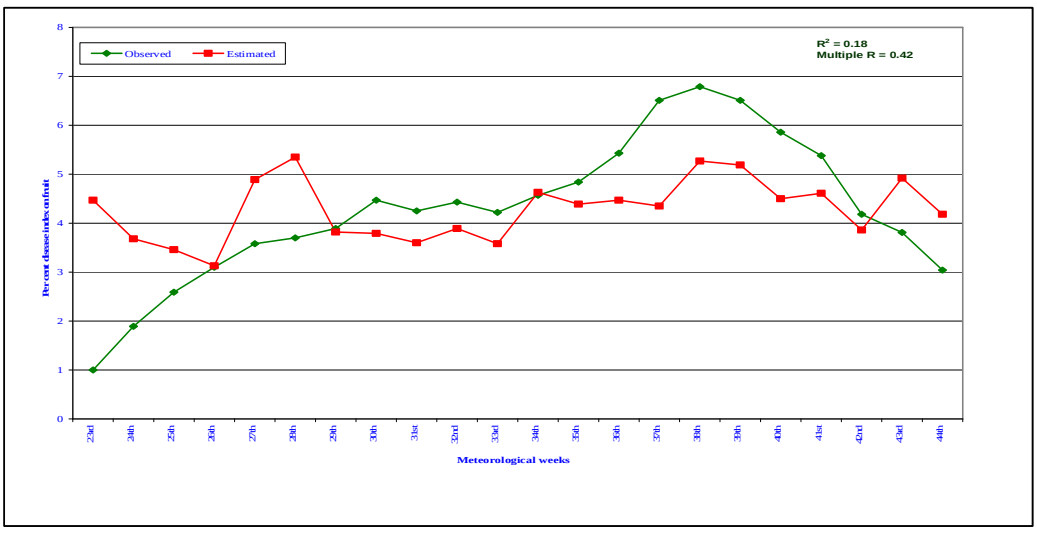


Fig. 14: Observed and estimated bacterial blight incidence and severity during Ambiabahar cropping season (April-2007 pruned)

of the disease on fruit (r values remained 0.187 and 0.257), but on leaf severity during the same season, rainfall had significant negative relationship. Similarly in ambiabahar cropping season, the disease severity on leaf and fruit followed the non-significant and positive correlation with rainfall (r values being 0.244 and 0.029, respectively) indicating that occurrence of rainfall irrespective of amount lead to increase in the amount of disease. On the reverse side, rainfall had significant negative relationship with all the components of the disease in mrigbahar cropping season. In hastbahar cropping season, rainfall had negative significant relationship with disease severity on leaf, but on fruits (incidence and severity) the effect of rainfall was non-significant.

Observed and estimated disease incidence and severity levels during different seasons revealed that, in late hastabahar cropping season (Table 18a), estimated disease incidence and severity on fruits was very close to the observed values ranged between 0.16 to 1.86 PDI and 0.09 to 1.68 PDI respectively (Fig. 13). On the contrary in ambiabahar cropping season (Table 18b), observed and estimated values of disease components followed the wider range between 0.17 to 2.99 (PDI on leaf), 0.00 to 2.02 (per cent incidence on fruit) and 0.03 to 3.47 (PDI on fruit) and were non-significantly correlated with weather factors (Fig. 14). During mrigbahar cropping season, observed disease severity on leaf was very close to the estimated disease severity with a narrow range between 0.14 to 1.78 PDI (Table 18c). Slight difference was recorded between the observed and estimated values of disease incidence (0.02 to 2.56%) and severity (0.00 to 2.26 PDI) on fruits (Fig. 15).

In hastabahar cropping season observed and estimated values of all the disease components followed the close path (Table 18d) ranged between 0.04 to 0.55 (PDI on leaf), 0.16 to 1.39 (per cent incidence on fruit) and 0.02 to 0.82 (PDI on fruit) indicating that prediction models are fit and reliable during the season (Fig. 16).

Combined effect of weather variables was found significantly favourable for bacterial blight development and spread in all the three seasons (except ambiabahar) as indicated by the significant coefficient values of multiple determination (R^2) ranged between 0.60 to 0.90 (Table 19) with an exception to this, in ambiabahar cropping season, overall effect of weather factors followed the non-significant positive relationship with disease development (R^2 values ranged between 0.17 – 0.40 on different disease components). Step-wise regression models developed between the disease development and weather variables revealed the strong significant relationship between the combined effect of weather factors and disease development with values (R^2) ranged between 0.17 to 0.90 indicating minimum of 17 per cent (PDI on leaf in ambiabahar season) to the maximum of 90 per cent (PDI on fruit during hastbahar) forecastability during different seasons.

4.8.2 Studies on survival of the pathogen

The survival of *X. axonopodis* pv. *punicae* in the infected leaf and fruit residues buried at different depths of sterilized and unsterilized soil was studied as per the method described in “Material and Methods”.

4.8.2.1 Survival of the pathogen in the infected leaf residues

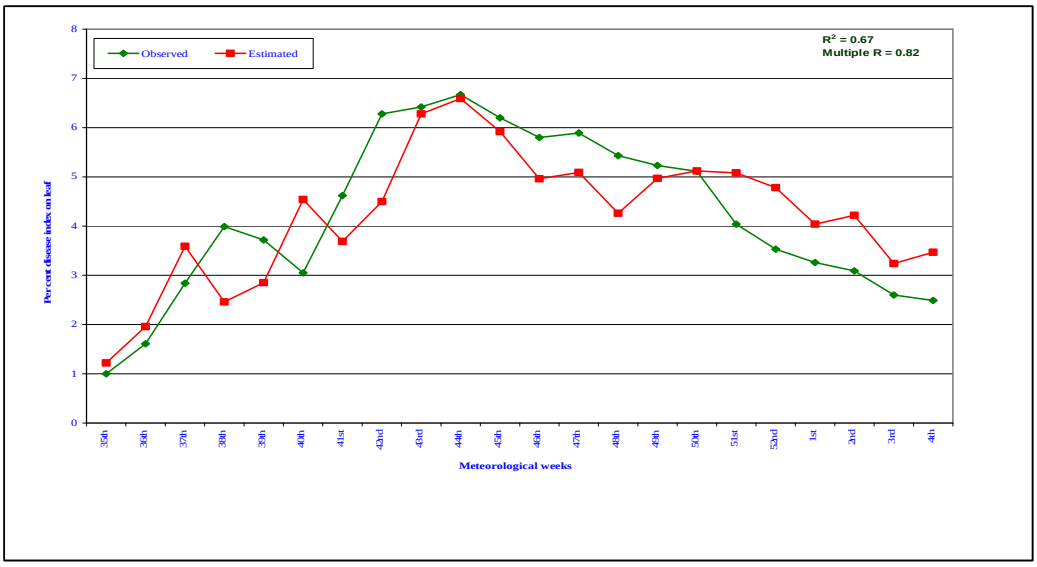
From the results obtained under sterilized soil condition (Table 20), the pathogen survived upto 20 weeks in the infected leaf residues kept on surface of the soil and also buried at 5 cm depth, but its survival period decreased as depth of burying the residues increased as evidenced by its survival upto 18th week and 16th week, when residues are buried at 10 cm and 15 cm, respectively (Fig. 17).

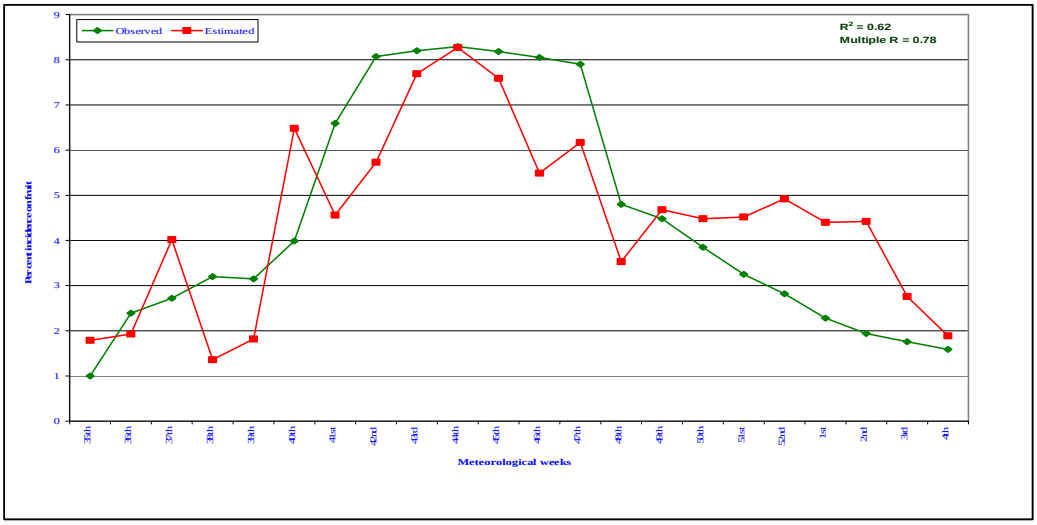
The population of the bacterium also declined as the period of burying increased irrespective of soil condition (sterilized/unsterilized) and type of residue material.

The bacterial colonies recovered through isolation after two weeks of burring on the surface of soil was estimated as 95.66×10^2 cfu per g and was gradually declined to 4×10^1 cfu per g of soil after 20th week of burying the material, whereas from leaf material buried at 5 cm depth, population recovered was 82.66×10^2 and 3.66×10^1 cfu per g of soil after 2nd and 20th week of isolation, respectively.

Table 18c: Observed and estimated bacterial blight incidence and severity during mrigbahar (July, 2007) cropping season

Standard meteorological weeks	Per cent disease index on leaf			Per cent incidence on fruit			Per cent disease index on fruit		
	Observed	Predicted	Difference	Observed	Predicted	Difference	Observed	Predicted	Difference
35th	1.00	1.22	-0.23	1.00	1.79	-0.79	1.00	1.59	-0.59
36th	1.61	1.96	-0.35	2.39	1.93	0.46	2.09	1.77	0.33
37th	2.84	3.59	-0.76	2.72	4.02	-1.30	2.46	3.28	-0.82
38th	3.99	2.46	1.53	3.20	1.36	1.84	2.92	1.37	1.55
39th	3.72	2.85	0.87	3.15	1.82	1.32	3.94	1.68	2.26
40th	3.05	4.54	-1.49	3.99	6.48	-2.49	3.44	4.91	-1.48
41st	4.62	3.69	0.93	6.59	4.56	2.02	4.13	3.57	0.56
42nd	6.28	4.50	1.78	8.07	5.73	2.34	6.12	4.59	1.54
43rd	6.42	6.28	0.14	8.20	7.69	0.51	6.41	5.96	0.45
44th	6.67	6.59	0.08	8.29	8.27	0.02	6.73	6.51	0.22
45th	6.20	5.92	0.27	8.18	7.59	0.58	6.64	5.83	0.81
46th	5.80	4.96	0.84	8.05	5.49	2.56	6.27	4.09	2.17
47th	5.89	5.09	0.80	7.90	6.17	1.73	5.70	4.62	1.08
48th	5.43	4.26	1.17	4.80	3.53	1.28	3.90	2.74	1.16
49th	5.23	4.97	0.26	4.48	4.68	-0.20	3.44	3.44	-0.00
50th	5.11	5.12	-0.02	3.85	4.48	-0.64	2.82	3.38	-0.56
51st	4.04	5.08	-1.04	3.25	4.52	-1.27	2.19	3.54	-1.35
52nd	3.53	4.78	-1.25	2.82	4.92	-2.10	1.33	3.89	-1.96
1st	3.26	4.04	-0.78	2.28	4.40	-2.12	1.89	3.41	-1.52
2nd	3.09	4.22	-1.14	1.94	4.42	-2.47	1.55	3.52	-1.97
3rd	2.60	3.24	-0.64	1.76	2.76	-0.97	1.11	2.42	-1.30
4th	2.49	3.47	-0.98	1.59	1.89	-0.32	1.09	1.66	-0.57





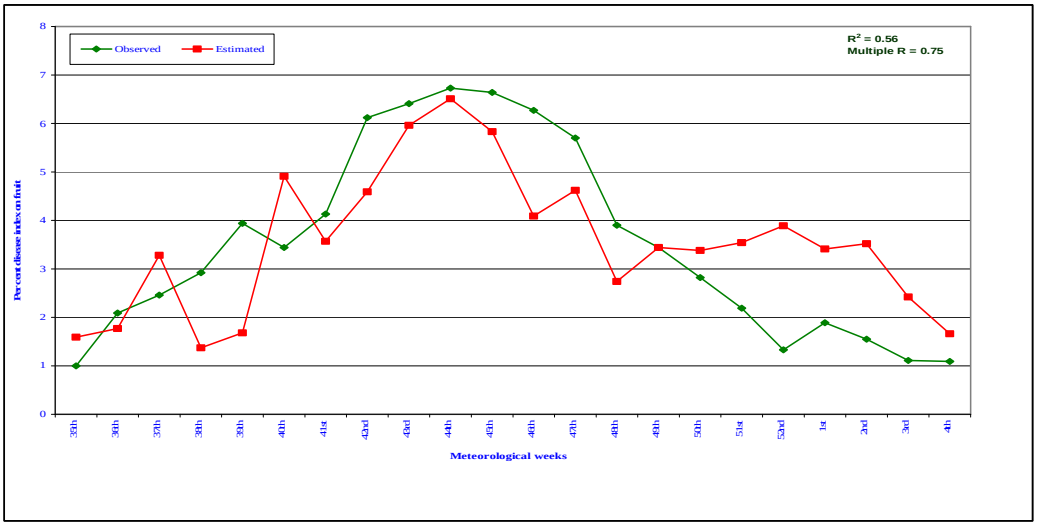
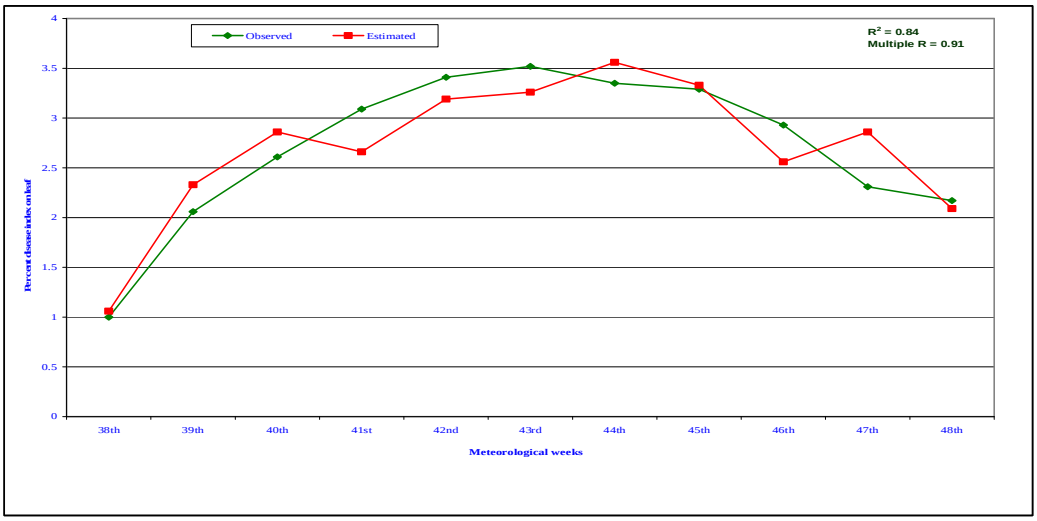
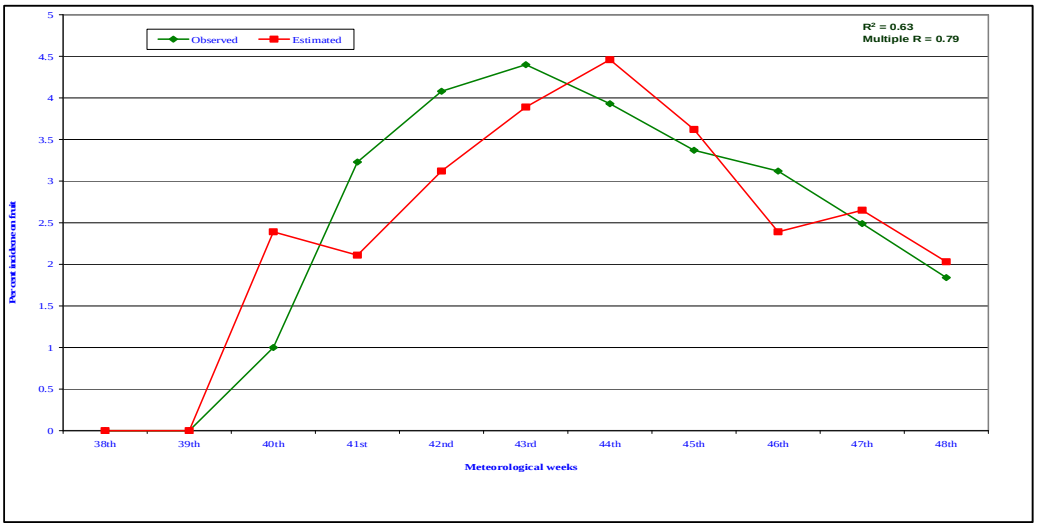


Fig. 15: Observed and estimated bacterial blight incidence and severity during Mrigbahar (July-2007 pruned) cropping season

Table 18d: Observed and estimated bacterial blight incidence and severity during hastbahar (September, 2007) cropping season

Standard meteorological weeks	Per cent disease index on leaf			Per cent incidence on fruit			Per cent disease index on fruit		
	Observed	Predicted	Difference	Observed	Predicted	Difference	Observed	Predicted	Difference
35th	-	-	-	-	-	-	-	-	-
36th	-	-	-	-	-	-	-	-	-
37th	-	-	-	-	-	-	-	-	-
38th	1.00	1.06	-0.06	-	-	-	-	-	-
39th	2.06	2.33	-0.26	-	-	-	-	-	-
40th	2.61	2.86	-0.25	1.00	2.39	1.39	1.00	1.82	-0.82
41st	3.09	2.66	0.43	3.23	2.11	1.13	2.16	1.55	0.61
42nd	3.41	3.19	0.22	4.08	3.12	0.96	2.90	2.44	0.47
43rd	3.52	3.26	0.27	4.40	3.89	0.51	3.36	3.38	-0.02
44th	3.35	3.56	-0.21	3.93	4.46	-0.54	3.69	3.86	-0.17
45th	3.29	3.33	-0.42	3.37	3.62	-0.25	3.12	3.03	0.09
46th	2.93	2.56	0.37	3.12	2.39	0.74	2.70	2.04	0.66
47th	2.31	2.86	-0.55	2.49	2.65	-0.16	1.95	2.18	-0.22
48th	2.17	2.09	0.79	1.84	2.03	-0.20	1.59	1.83	-0.25
49th	-	-	-	-	-	-	-	-	-





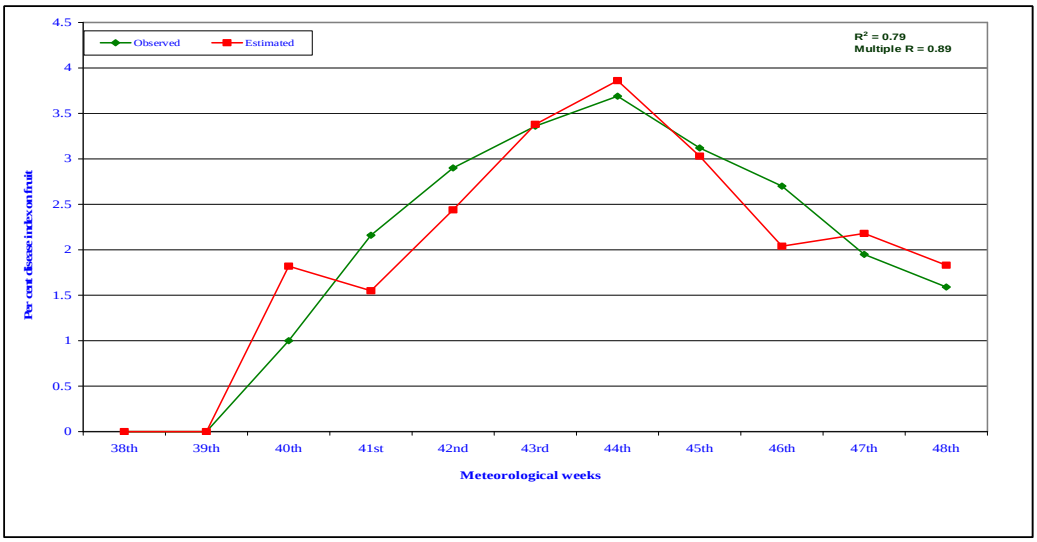


Fig. 16: Observed and estimated bacterial blight incidence and severity during Hastbahar (September-2007 pruned) cropping season

Table 19: Multiple regression models for bacterial blight of pomegranate in relation to weather factors during different seasons of 2006 and 2007

Cropping seasons	Coefficient of multiple determination (R^2)	Regression models
November 2006		
PDI on leaf	0.66*	$Y = 640.27 - 1.57 (X_1) - 1.60 (X_2) - 1.99 (X_3) - 7.98 (X_4)$
Per cent incidence on fruit	0.78*	$Y = 490.03 + 2.00 (X_1) - 1.612 (X_2) - 1.510 (X_3) + 3.98 (X_4)$
PDI on fruit	0.89**	$Y = 689.16 - 2.69 (X_1) - 1.45 (X_2) - 2.24 (X_3) + 2.24 (X_4)$
April 2007		
PDI on leaf	0.17 NS	$Y = 58.68 + 8.57 (X_1) - 2.38 (X_2) - 2.56 (X_3) + 1.57 (X_4)$
Per cent incidence on fruit	0.40 NS	$Y = 166.13 + 5.26 (X_1) - 3.62 (X_2) + 1.47 (X_3) + 5.93 (X_4)$
PDI on fruit	0.30 NS	$Y = 123.93 + 3.86 (X_1) - 2.99 (X_2) + 1.08 (X_3) + 4.78 (X_4)$
July 2007		
PDI on leaf	0.65*	$Y = 187.78 - 3.35 (X_1) - 6.10 (X_2) - 6.66 (X_3) - 2.23 (X_4)$
Per cent incidence on fruit	0.61*	$Y = 422.99 - 9.41 (X_1) + 1.14 (X_2) - 1.53 (X_3) - 4.46 (X_4)$
PDI on fruit	0.60*	$Y = 256.40 - 5.44 (X_1) + 7.58 (X_2) - 1.05 (X_3) - 2.58 (X_4)$
September 2007		
PDI on leaf	0.80*	$Y = 56.77 - 1.22 (X_1) + 5.72 (X_2) - 2.76 (X_3) - 1.97 (X_4)$
Per cent incidence on fruit	0.74 NS	$Y = 87.92 - 1.59 (X_1) + 1.35 (X_2) - 4.39 (X_3) + 7.42 (X_4)$
PDI on fruit	0.90**	$Y = 68.46 - 1.21 (X_1) - 1.29 (X_2) - 3.22 (X_3) + 3.07 (X_4)$

Y = Disease incidence/disease index
X_1 = Maximum temperature
X_2 = Minimum temperature
X_3 = Relative humidity
X_4 = Rainfall

** : Significant at 1% level
* : Significant at 5% level

At 10 and 15 cm depth, the recovery of the bacterium was highest in the beginning after 2nd week (106.66×10^2 cfu/g and 89.33×10^2 cfu/g, respectively) later on came down to 2×10^1 cfu per g (10 cm) and 3.33×10^2 cfu per g after 18th and 16th week, respectively.

Under unsterilized soil condition, bacterium survived for the lesser period than in sterilized soil condition. It survived upto 16th week in the leaf residue present on the surface of the soil, but survived upto 18th week at 5 cm depth (4.33×10^2 cfu/g). At 10th and 15th cm

Table 20: Survival period of *Xanthomonas axonopodis* pv. *punicae* in the infected leaf residues burried in sterilized and unsterilized soil condition

Sl. No.	Survival period (weeks)	Population of the bacterium (cfu/g of soil) at different depths							
		Sterilized soil condition (cm depth)				Unsterilized soil condition (cm depth)			
		Surface of soil	5	10	15	Surface of soil	5	10	15
1.	2	95.66×10^2	82.66×10^2	106.66×10^2	89.33×10^2	108.33×10^2	98.00×10^2	85.33×10^2	73.00×10^2
2.	4	79.33×10^2	69.00×10^2	101.00×10^2	73.33×10^2	85.00×10^2	75.33×10^2	62.66×10^2	56.00×10^2
3.	6	63.00×10^2	58.66×10^2	81.33×10^2	56.00×10^2	72.00×10^2	61.33×10^2	50.00×10^2	37.66×10^2
4.	8	51.66×10^2	43.00×10^2	64.00×10^2	42.00×10^2	63.66×10^2	46.66×10^2	37.66×10^2	23.33×10^2
5.	10	45.33×10^2	32.33×10^2	36.00×10^2	25.66×10^2	33.66×10^2	29.66×10^2	32.00×10^2	14.33×10^2
6.	12	39.33×10^2	23.66×10^2	19.67×10^2	15.00×10^2	19.00×10^2	21.00×10^2	18.00×10^2	4.67×10^2
7.	14	25.66×10^2	16.00×10^2	13.00×10^2	6.00×10^2	11.00×10^2	16.33×10^2	10.00×10^2	2.00×10^2
8.	16	21.00×10^2	11.00×10^2	7.66×10^2	3.33×10^2	5.33×10^2	8.66×10^2	3.00×10^2	-
9.	18	9.66×10^2	6.00×10^2	2.00×10^1	-	-	4.33×10^2	-	-
10.	20	4.00×10^1	3.66×10^1	-	-	-	-	-	-
11.	22	-	-	-	-	-	-	-	-

Population count is taken 72 hours after incubation

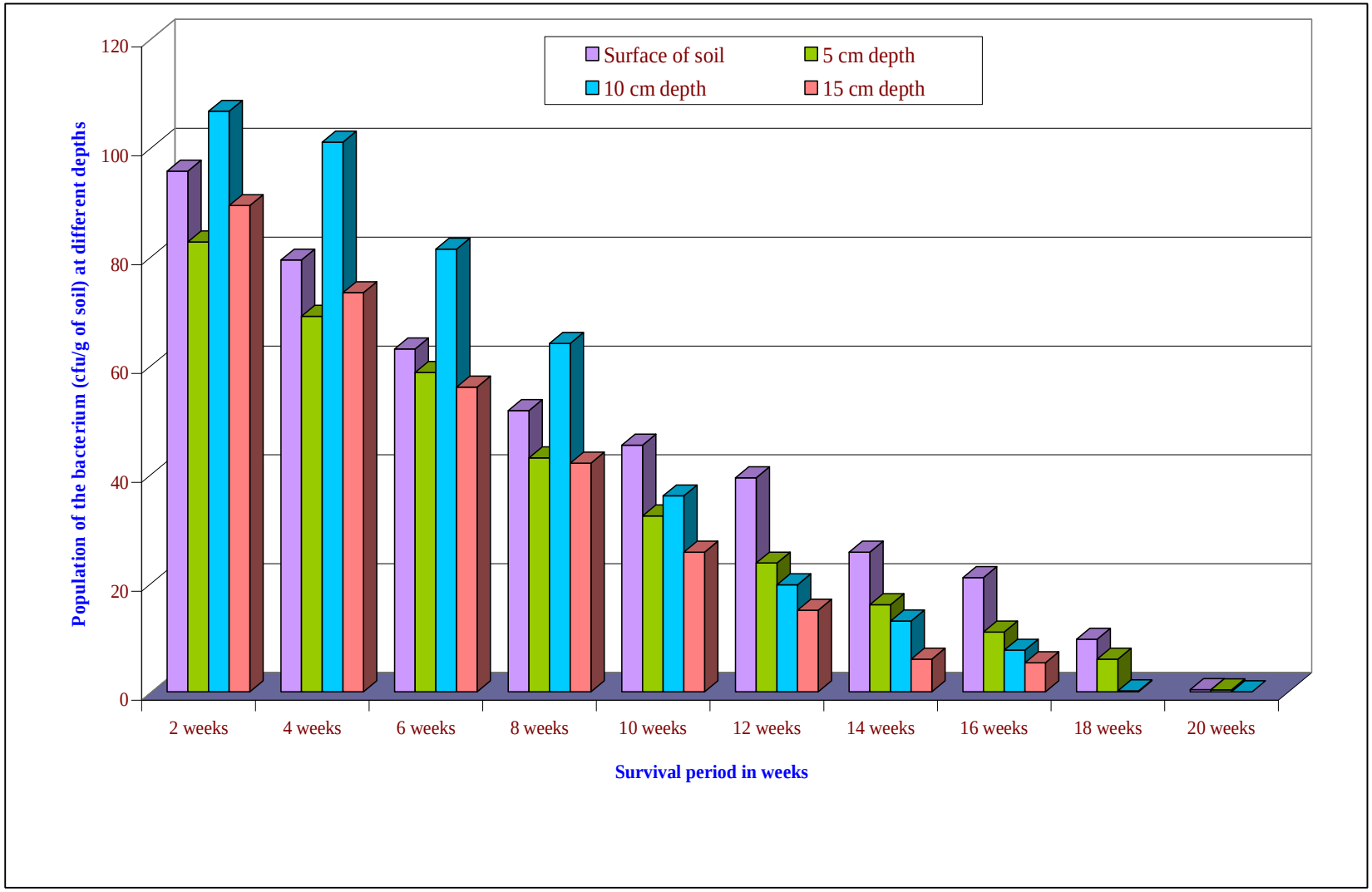


Fig. 17: Survival period of Xanthomonas axonopodis pv. punicae in the infected leaf residues buried in sterilized soil condition

depth, bacterium was isolated upto 16th (population was 3.00 × 10^2 cfu/g) and 14th week (2.00 × 10^2 cfu/g), respectively (Fig. 18).

4.8.2.2 Survival of the pathogen in the infected fruit residue

Similarly, in the infected fruit residues buried at different depths of soil, pathogen survived upto 20 and 22 weeks under sterilized soil condition and upto 18 to 20 weeks under unsterilized soil condition. Results are presented in Table 21.

Under sterilized soil condition (Fig. 19), bacterium was isolated from 2nd to 22nd week with a population ranged from 177.00 × 10^2 cfu per g to 3.33 × 10^1 cfu per g of soil, respectively in fruit residue kept on surface of the soil, whereas it was recovered with a population of 159.00 × 10^2 cfu per g (in the beginning after 2nd week) and 1.66 × 10^1 cfu per g (after 22nd week) at 5 cm depth. The population of the pathogen decreased from 135.67 x 10^2 cfu/g to 3.33 x 10^1 cfu/g from 2nd to 20th week at 10 cm depth and from 106.33 x 10^2 to 2.66 x 10^1 cfu/g per gram of soil at 15 cm depth.

Under unsterilized soil condition survival period of the pathogen was found less and it survived upto 20th week at 5 cm depth (2.33 × 10^1 cfu/g) and on the surface of soil (4.00 × 10^1 cfu/g) and upto 18 weeks when infected fruit residues were buried at 10 and 15 cm depths. The population varied from 2nd to 18th week in a range of 118 × 10^2 to 4.33 × 10^2 cfu per g and 94.33 × 10^2 to 3.00 × 10^1 cfu per g at 10 cm and 15 cm depth, respectively under unsterilized soil condition (Fig. 20).

4.8.3 Studies on host range of *Xanthomonas axonopodis* pv. *punicae*

The ability of the pathogen to infect ten hosts was studied by inoculating the culture suspension of the pathogen under artificial conditions. A total of eight weeds and two perennial plants such as neem and bael were inoculated. The results of the study are presented in Table 22.

It was observed that, among the ten plant species tried, the pathogen could infect neem, tridax and achyranthus in addition to its main host pomegranate (Plate 10). Disease symptoms on these plants appeared after 10 to 12 days of inoculation. On neem plant, symptoms appeared as white coloured, water soaked lesions in the beginning, later on turned to brown to black coloured spots lead to the blightining of the leaf lamina. Achyranthes showed circular/irregular brown to black coloured spots on the leaves. Symptoms on Tridax appeared as water soaked lesions in the beginning later on turned to black coloured spots. Necrosis of the tissue was also observed at later stages. Reisolation and cross inoculation studies confirmed the host susceptibility.

4.9 Integrated disease management

4.9.1 Efficacy of bleaching powder and Bordeaux mixture in reducing the initial inoculum of the pathogen

The investigation was carried out to assess the effectiveness of bleaching powder and Bordeaux mixture against bacterial blight pathogen as described in “Material and Methods”.

The results of the study revealed that, Bordeaux mixture (1% spray) alone (Table 23) or in combination with bleaching powder (soil application) was significantly effective in reducing the pathogen population as there was no recovery of the pathogen from treated foliage upon isolation. The next best effective treatment was copper oxychloride (0.2%) with bleaching powder @ 100 g per plant (spray and soil application, respectively), which significantly recorded the least number of bacterial colonies (18.25 cfu/ml), copper oxychloride (0.2%) and bleaching powder (100 g/plant) as the separate treatments were found moderately effective and non-significant with each other in reducing the pathogen population. Highest recovery of the bacterium (43.75 cfu/ml) was obtained from untreated foliage (Fig. 21 and Plate 11).

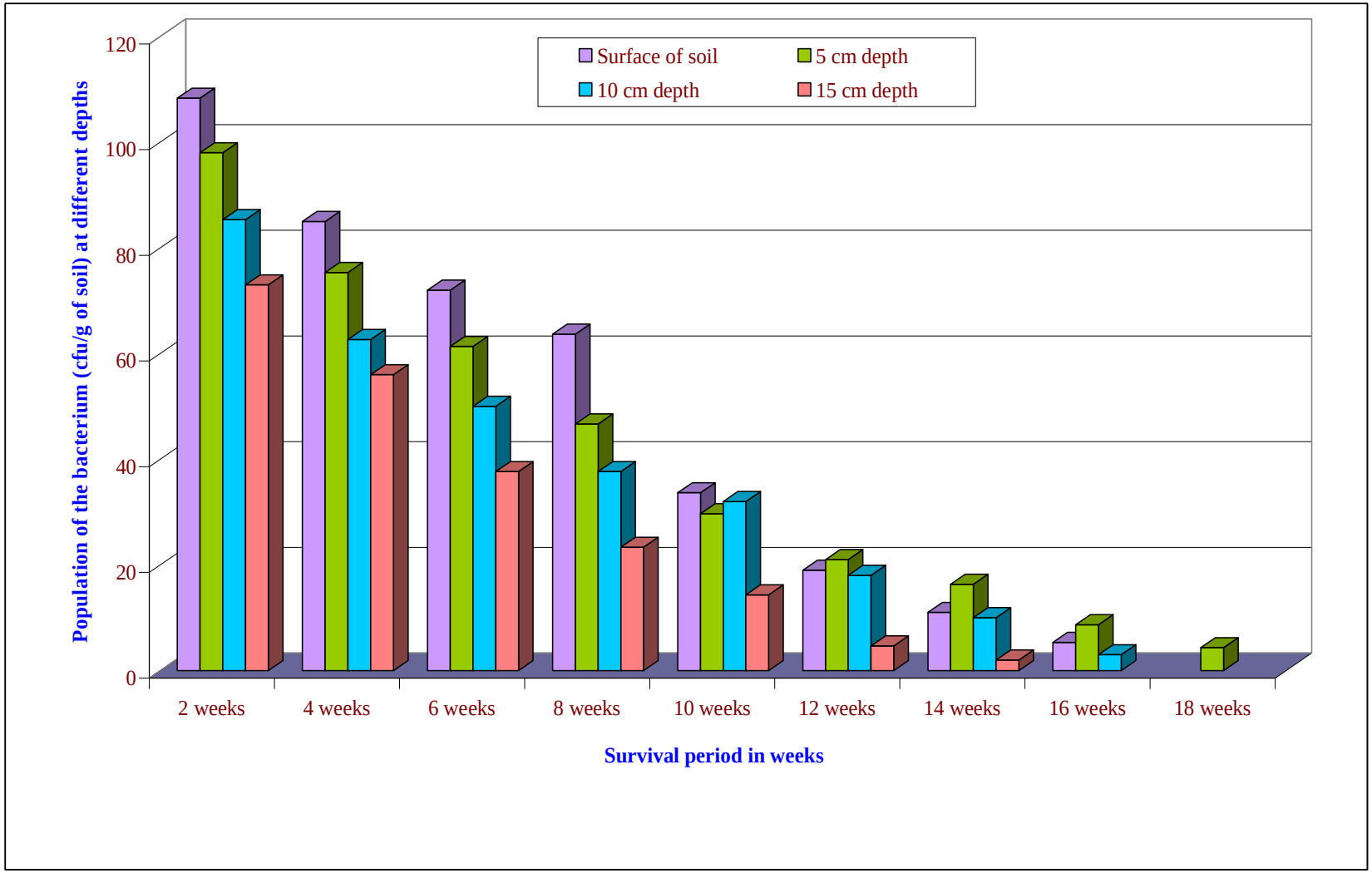


Fig. 18: Survival period of Xanthomonas axonopodis pv. punicae in the infected leaf residues buried in unsterilized soil condition

Table 21: Survival period of *Xanthomonas axonopodis* pv. *punicae* in the infected fruit residues buried in sterilized and unsterilized soil condition

Sl. No.	Survival period (weeks)	Population of the bacterium (cfu/g of soil) at different depths							
		Sterilized soil condition (cm depth)				Unsterilized soil condition (cm depth)			
		Surface of soil	5	10	15	Surface of soil	5	10	15
1.	2	177.00×10^2	159.00×10^2	135.67×10^2	106.33×10^2	201.63×10^2	160.67×10^2	118.00×10^2	94.33×10^2
2.	4	169.66×10^2	142.66×10^2	117.00×10^2	98.33×10^2	192.00×10^2	155.00×10^2	99.33×10^2	89.00×10^2
3.	6	132.67×10^2	119.00×10^2	97.33×10^2	80.33×10^2	166.66×10^2	136.00×10^2	89.33×10^2	72.66×10^2
4.	8	95.33×10^2	90.00×10^2	85.00×10^2	65.67×10^2	97.00×10^2	88.33×10^2	75.33×10^2	57.66×10^2
5.	10	86.33×10^2	73.00×10^2	62.67×10^2	42.33×10^2	75.00×10^2	64.00×10^2	46.33×10^2	38.67×10^2
6.	12	57.33×10^2	48.33×10^2	40.00×10^2	29.33×10^2	52.00×10^2	42.33×10^2	29.00×10^2	21.00×10^2
7.	14	39.00×10^2	36.33×10^2	24.33×10^2	13.00×10^2	31.00×10^2	22.00×10^2	14.00×10^2	11.33×10^2
8.	16	29.00×10^2	27.00×10^2	11.33×10^2	6.67×10^2	16.00×10^2	10.66×10^2	8.00×10^2	5.66×10^2
9.	18	15.33×10^2	13.00×10^2	6.66×10^2	4.00×10^1	9.66×10^2	4.00×10^2	4.33×10^2	3.00×10^1
10.	20	7.00×10^2	5.33×10^2	3.33×10^1	2.66×10^1	4.00×10^1	2.33×10^1	-	-
11.	22	3.33×10^1	1.66×10^1	-	-	-	-	-	-
12.	24	-	-	-	-	-	-	-	-
13.	26	-	-	-	-	-	-	-	-

Population count is taken 72 hours after incubation

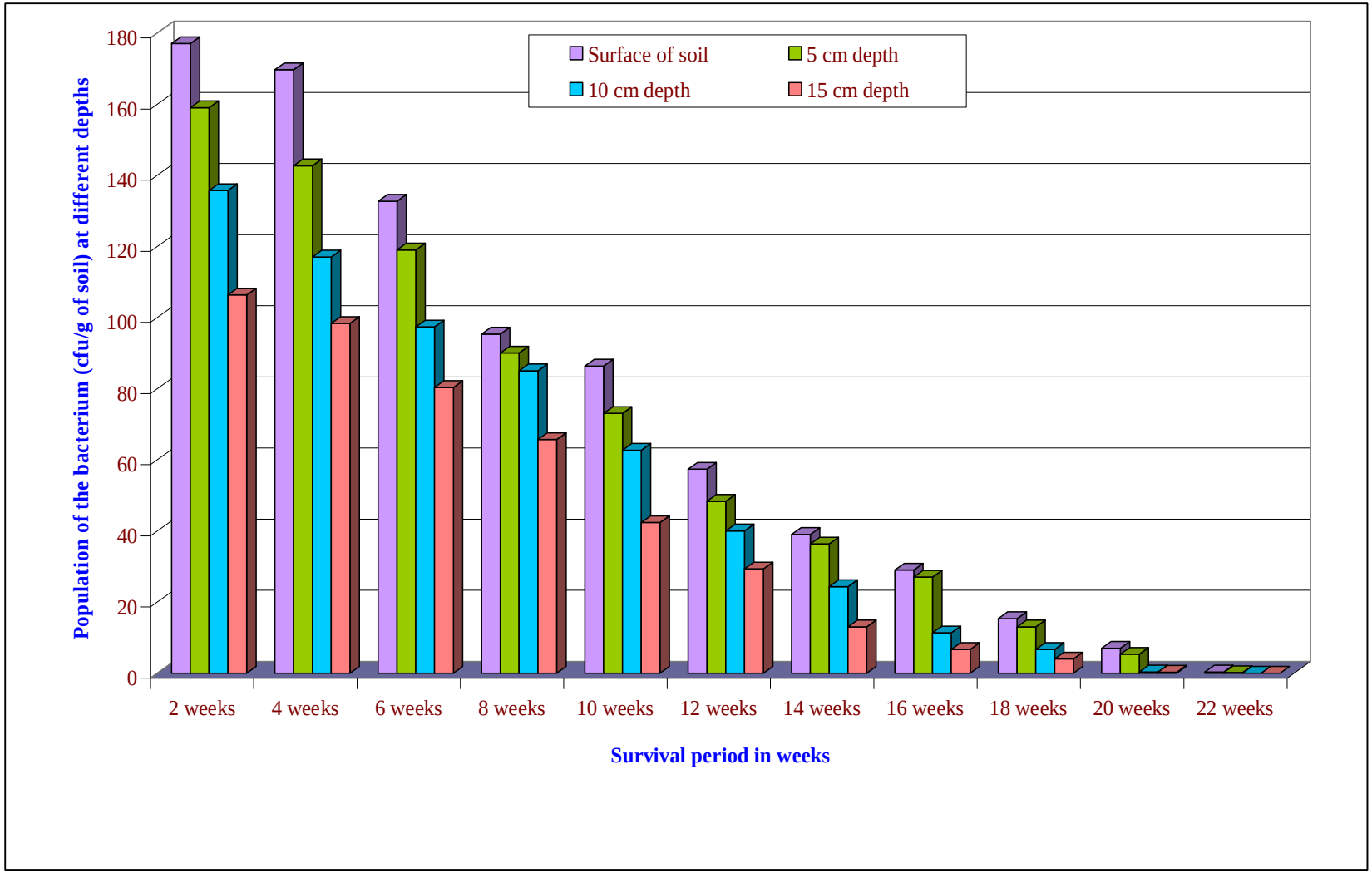


Fig. 19: Survival period of Xanthomonas axonopodis pv. punicae in the infected fruit residues buried in sterilized soil condition

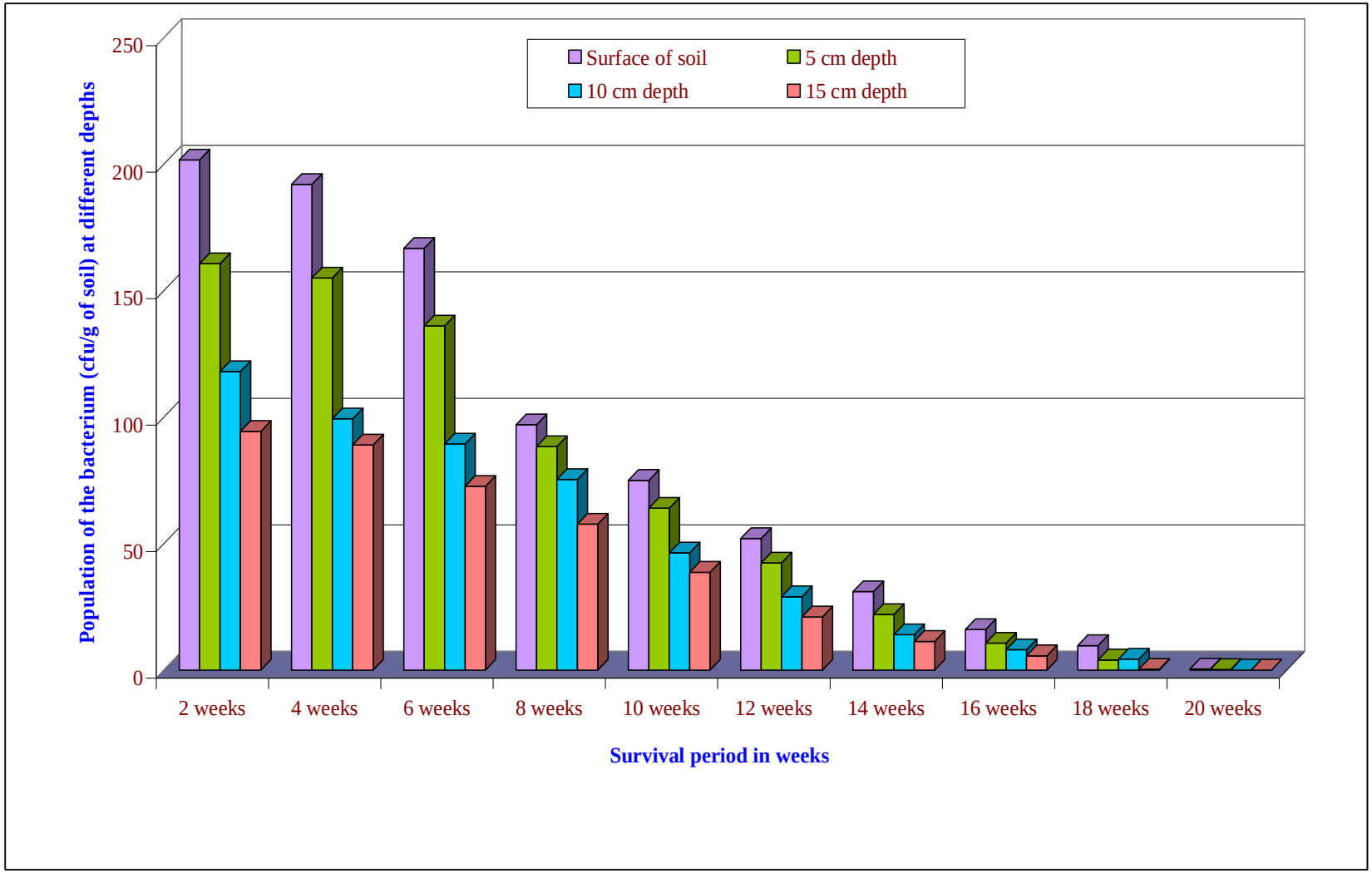


Fig. 20: Survival period of Xanthomonas axonopodis pv. punicae in the infected fruit residues buried in unsterilized soil condition

Table 22: Host range of *Xanthomonas axonopodis* pv. *punicae*

Sl. No.	Common name	Scientific name	Reaction	Symptoms
1.	Parthenium	*Parthenium hysterophorus* L.	-	-
2.	Neem	*Azadirachta indica* A. Juss	+	Appeared as water soaked lesions in the beginning later on turned to brown to black coloured spots, lead to the blighting of leaf lamina
3.	Sida	*Sida cordifolia* L.	-	-
4.	Tridax	*Tridax procumbens* L.	+	Water soaked lesions, later turned to black coloured spots, which lead to the tissue necrosis.
5.	Achyaranthes	*Achyranthes aspera* L.	+	Circular to irregular brown to black coloured spots were noticed on the leaves
6.	Croton	*Croton sparsiflorus* Morong	-	-
7.	Bael	*Aegle marmelos* L.	-	-
8.	Physalis	*Physalis minima* L.	-	-
9.	Tinospora	*Tinospora cordifolia* (Willd) Miers	-	-
10.	Euphorbia	*Euphorbia pulcherima* (Willd) Exklotzsch.	-	-

4.9.2 Effect of different bactericides on stem treatment against the spread of bacterial blight in pomegranate

Infected stem or branches serve as a primary source of inoculum, through which bacterial blight spreads from one season to another. Eradication of stem infection by mechanical means is often difficult. Hence, an experiment was carried out to know the efficacy of different bactericides (in combination with copper oxychloride) and antibacterial chemicals by the way of pasting these chemicals on stem and branches of the plant after pruning of the crop (Plate 12). Methodology is furnished in “Material and Methods”. The antibiotics/bactericides were used at the concentration of 0.05% each in combination with copper oxychloride @ 0.2% (Table 24).

It was observed that, crop was completely free from the disease upto one month after pasting irrespective of treatments. Observations on disease recorded two months after pasting indicated that, black out (18.52%) followed by bronip (20.16%) and bacterinashak (20.24%) each in combination with copper oxychloride, significantly recorded the lowest bacterial blight incidence, whereas disease severity was significantly lowest in bronip + COC (5.79 PDI) followed by streptocycline + COC (7.76 PDI) treated plot, which were at par with each other and significantly superior over other treatments of the trial. The next best effective treatment in reducing the disease severity was black out, followed by bactinash 200 and bacterinashak in combination with copper oxychloride.

Similarly, results recorded on 90 days after pasting showed that, black out and bronip each in combination with COC were found significantly very effective by recording the least bacterial blight incidence of 24.90 and 30.80 per cent, respectively. But, in respect of reducing the disease severity, bronip (9.95 PDI) and streptocycline (12.68 PDI) (each in combination

Azadirachta indica

Achyranthes aspera

Tridax procumbens

Plate 10: Host range of X. axonopodis pv.punicae

Table 23: Efficacy of bleaching powder and copper compounds in reducing the initial inoculum of *Xanthomonas axonopodis* pv. *punicae*

Sl. No.	Treatment details	Concentration	Recovery of the pathogen from treated foliage (cfu/ml)
1.	Bleaching powder	100 g/plant	27.50 (5.33)*
2.	Bordeux mixture	1%	0.00 (1.00)
3.	Bordeux mixture + bleaching powder	1% + 100 g/plant	0.00 (1.00)
4.	Copper oxychloride	0.2%	30.25 (5.58)
5.	Copper oxychloride + bleaching powder	0.2% + 100 g/plant	18.25 (4.38)
6.	Untreated control	-	43.75 (6.67)
	SEm±	-	0.17
	CD at 1%	-	0.70

* $\sqrt{x+1}$ transformed values

with COC) were found significantly very effective than other treatments of the trial. The other treatments such as bactrinashak + COC and bactinash 200 + COC found moderately effective and on par with each other in reducing incidence and severity of the disease. Pasting with copper oxychloride was found least effective in controlling the disease. Highest disease incidence (62.17 PDI) and severity (31.99 PDI) were recorded in untreated check plot (Fig. 22).

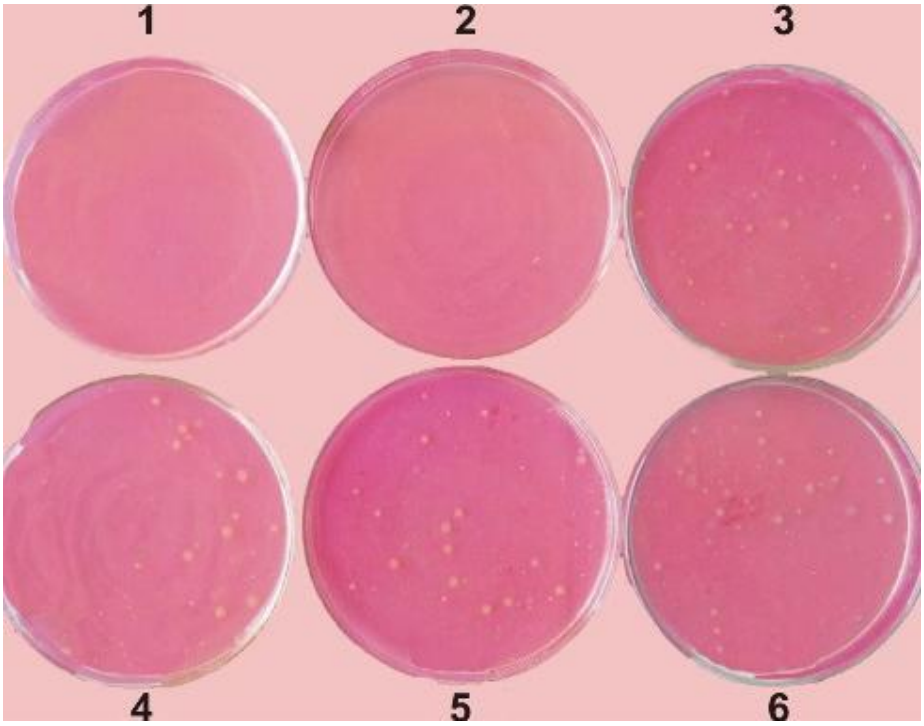
Looking into the yield, highest yield of 9.81 and 8.43 tonnes per ha was recorded in black out + COC followed by bronip + COC treated plots, respectively. Lowest yield of 2.65 tonnes per ha was obtained in untreated check plot.

4.9.3 Evaluation of bactericides/antibacterial chemicals

4.9.3.1 *In vitro* evaluation of bactericides/antibacterial chemicals

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

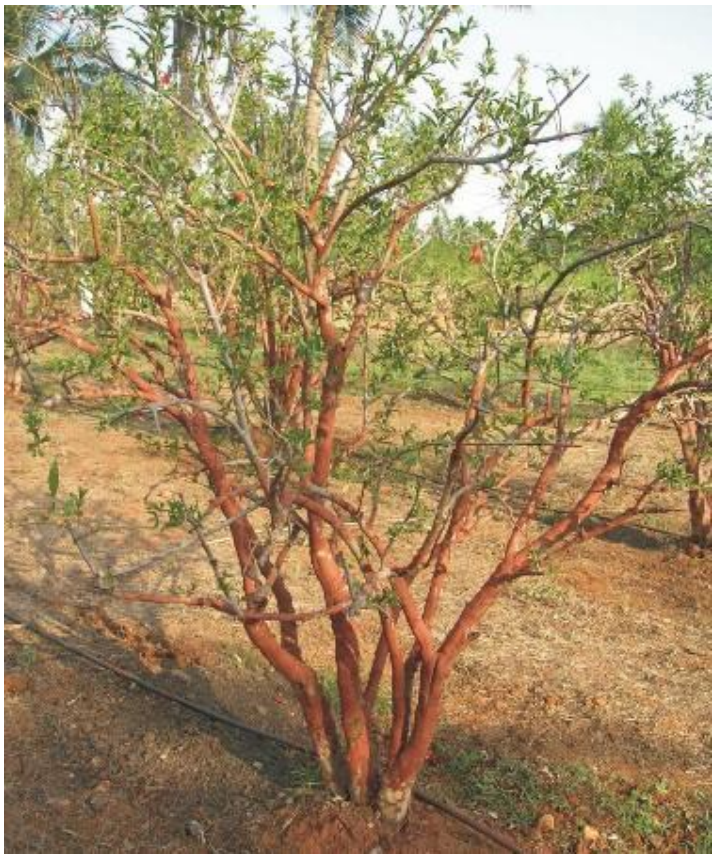
Application of bleaching powder to the basin of the plant during rest period



Recovery of colonies of X. axonopodis pv. punicae in different treatments

Plate 11: Efficacy of bleaching powder and copper compounds in reducing the initial inoculum of X. axomopodis pv. punicae

Field view



Individual plant

Plate 12: Stem treatment of bronip (0.05%) + copper oxychloride (0.2%)

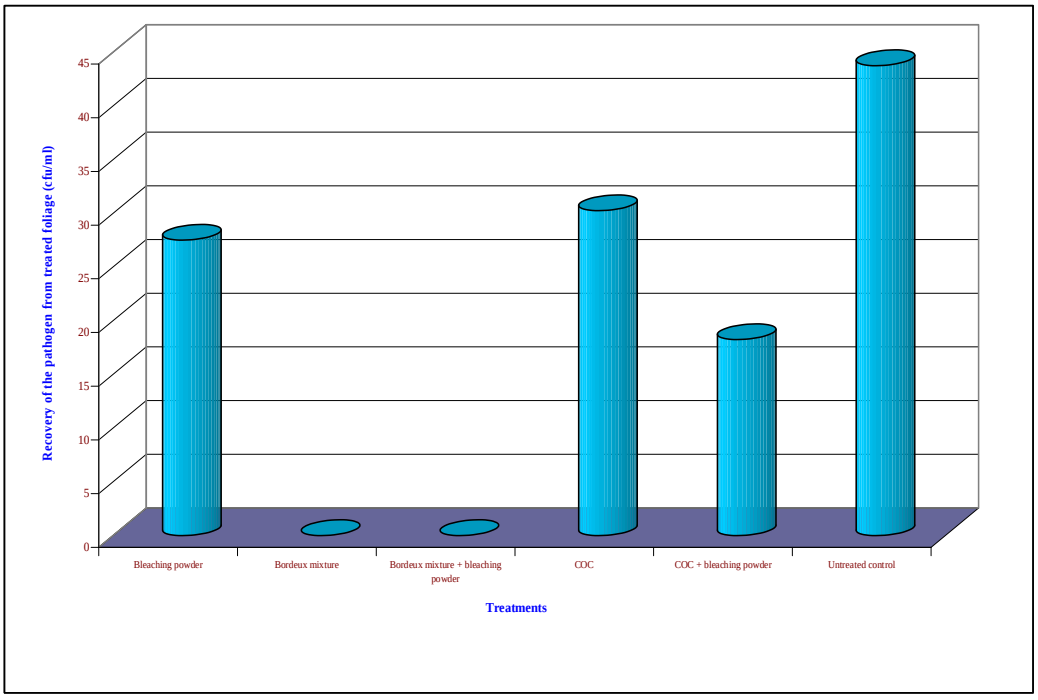


Fig. 21: Efficacy of bleaching powder and copper compounds in reducing the initial inoculum ofXanthomonas axonopodis pv. punicae

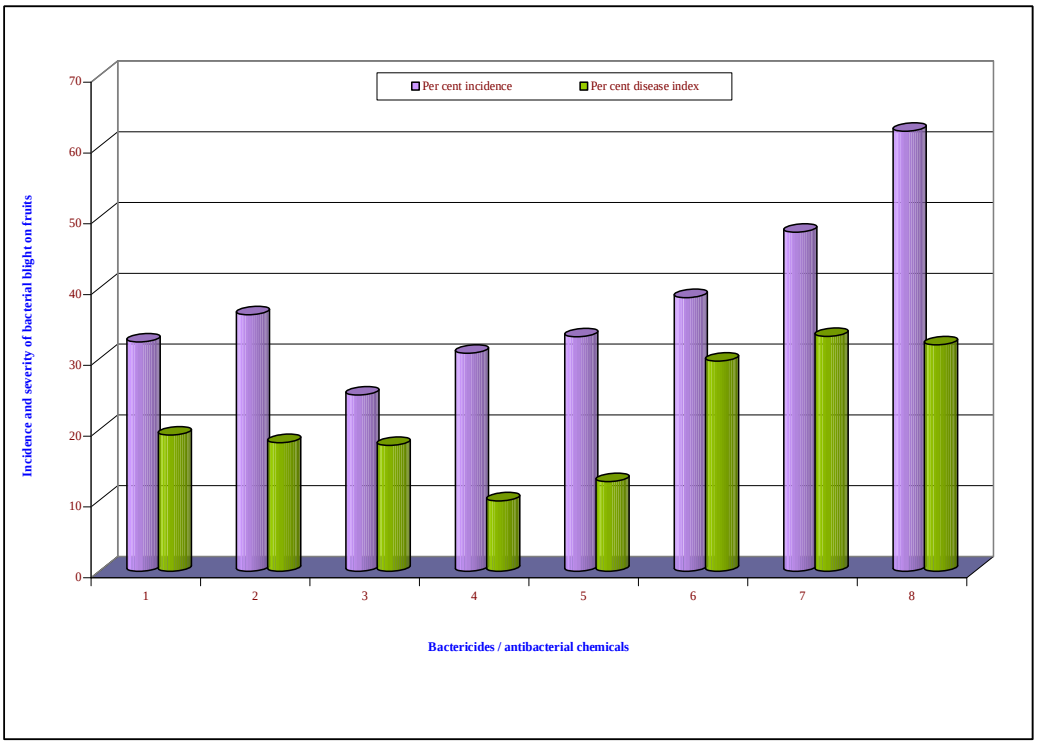


Fig. 22: Effect of stem pasting of bactericides/antibacterial chemicals on the incidence and severity of bacterial blight of pomegranate caused by Xanthomonas axonopodis pv. punicae(90 days after pasting)

Table 24: Effect of stem pasting of bactericides/antibacterial chemicals on the incidence and severity of bacterial blight of pomegranate caused by *Xanthomonas axonopodis pv. punicae*

Sl. No.	Bactericides / antibacterial chemicals	Concentration (%)	Incidence and severity of bacterial blight on fruits at						
			60 days after pasting		90 days after pasting		Yield		Per cent increase in yield over untreated control
			Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	kg/plant	tonnes/ha	
1.	Bactrinashak + Copper oxychloride	0.05 0.2	20.24 (26.70)	13.47 (21.48)	32.40 (34.67)	19.24 (25.95)	16.24	8.12	206.42
2.	Bactinash-200 + Copper oxychloride	0.05 0.2	25.42 (30.19)	11.77 (20.01)	36.25 (36.99)	18.18 (25.22)	15.17	7.59	186.42
3.	Black out + Copper oxychloride	0.05 0.2	18.52 (25.38)	10.78 (19.14)	24.90 (29.89)	17.79 (24.88)	19.61	9.81	270.19
4.	Bronip + Copper oxychloride	0.05 0.2	20.16 (26.67)	5.79 (13.81)	30.80 (33.69)	9.95 (18.30)	16.86	8.43	218.11
5.	Streptocycline + Copper oxychloride	0.05 0.2	24.86 (29.88)	7.76 (16.03)	33.13 (35.11)	12.68 (20.74)	14.39	7.20	171.70
6.	Bordeux mixture	1	29.19 (32.69)	16.45 (23.76)	38.67 (38.46)	29.69 (32.97)	13.04	6.52	146.04
7.	Copper oxychloride	0.2	38.35 (38.23)	21.34 (27.45)	47.91 (43.79)	33.19 (35.14)	9.43	4.72	78.11
8.	Untreated control	-	43.89 (41.49)	23.47 (28.90)	62.17 (52.06)	31.99 (34.36)	5.30	2.65	-
	SEm± CD at 5%		1.49 4.51	1.44 4.37	1.44 4.39	1.33 4.05	1.25 3.79	0.62 1.89	- -

Figures in parentheses are angular transformed values
Variety : Bhagwa Date of pruning : 10-15 March, 2008
Cropping season : Ambiabahar – 2008-09 Date of pasting : 20 days after pruning
Incidence and severity of bacterial blight at the time of pasting and one month after pasting : Nil

Table 25: *In vitro* evaluation of bactericides/antibacterial chemicals on the growth of *Xanthomonas axonopodis* pv. *punicae*

Sl. No.	Bactericides	Inhibition zone (mean diameter in mm)		
		0.05%	0.1%	Mean
1.	Bactinash 200	12.86 (3.71)#	17.27 (4.27)	15.07 (3.99)
2.	Bactrinashak	7.66 (2.94)	10.10 (3.32)	8.88 (3.13)
3.	Bronip	11.67 (3.56)	17.67 (4.32)	14.67 (3.94)
4.	K-cycline	8.90 (3.14)	11.56 (3.54)	10.23 (3.34)
5.	Streptocycline	7.47 (2.91)	9.33 (3.21)	8.40 (3.06)
	Antibacterial chemicals	0.5%	1.0%	Mean
6.	Bleaching powder	7.73 (2.95)	9.73 (3.27)	8.73 (3.11)
7.	Bordeux mixture	7.57 (2.93)	10.47 (3.38)	9.02 (3.16)
8.	Copper oxychloride	7.50* (2.92)	11.97** (3.58)	9.74 (3.25)
9.	Plantomycin	11.30 (3.51)	16.23 (4.14)	13.77 (3.83)
10.	Untreated control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	Mean	8.27 (2.96)	11.43 (3.40)	9.85 (3.18)
	Source	SEm		CD at 1%
	Bactericides (B)	0.093		0.35
	Concentration C)	0.041		0.16
	Interaction (B × C)	0.13		0.50

$\sqrt{x+1}$ transformed values
* : Copper oxychloride @ 0.2% ** : Copper oxychloride @ 0.3%

Results indicated that (Table 25), out of six bactericides and three antibacterial chemicals evaluated, significantly superior efficacy was exhibited by bactinash-200 (Fig. 23 and 24) with an inhibition zone of 15.07 mm followed by bronip (14.67 mm) and plantomycin (13.77 mm). However, both the treatments were on par with each other. All other bactericides antibacterial chemicals *viz.*, K-cycline (10.23 mm), copper oxychloride (9.74 mm), Bordeaux mixture (9.02 mm), bactrinashak (8.88 mm), bleaching powder (8.73 mm) and streptocycline (8.40 mm) were found moderately effective and remained on par with each other (Plate 13).

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

Interaction effect among the bactericides and concentration indicated that, bronip and bactinash-200 @ 0.1% concentration were highly effective with an inhibition zone of 17.67 mm and 17.27 mm, respectively followed by plantomycin @ 1% concentration (16.23 mm).

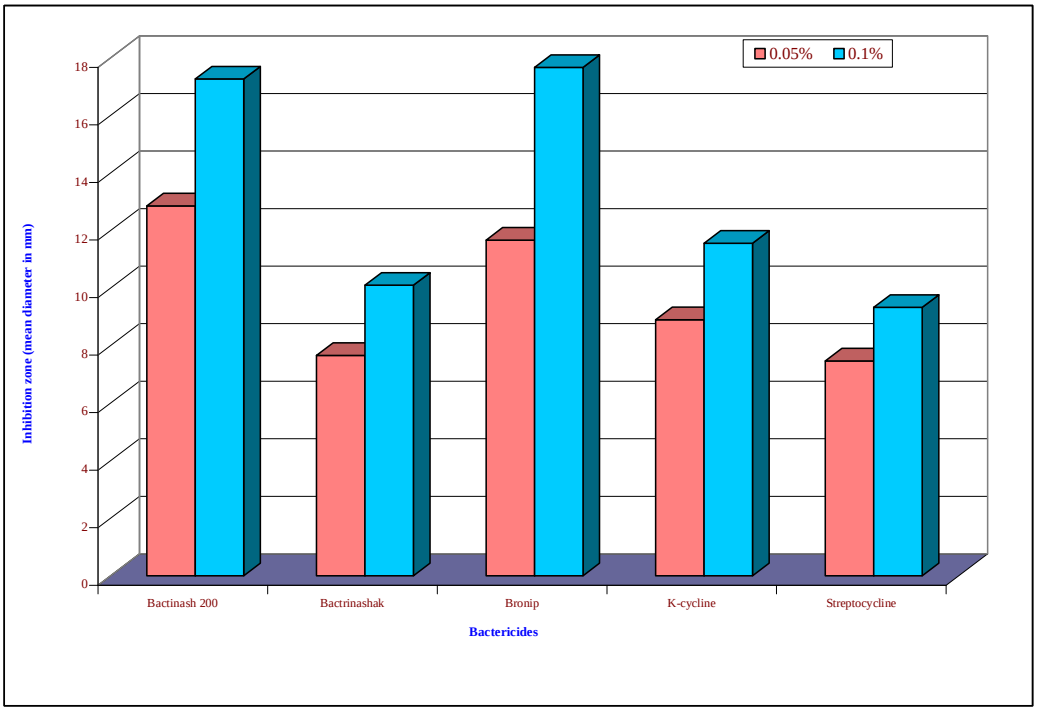


Fig. 23: In vitro evaluation of bactericides on the growth of Xanthomonas axonopodis pv. punicae

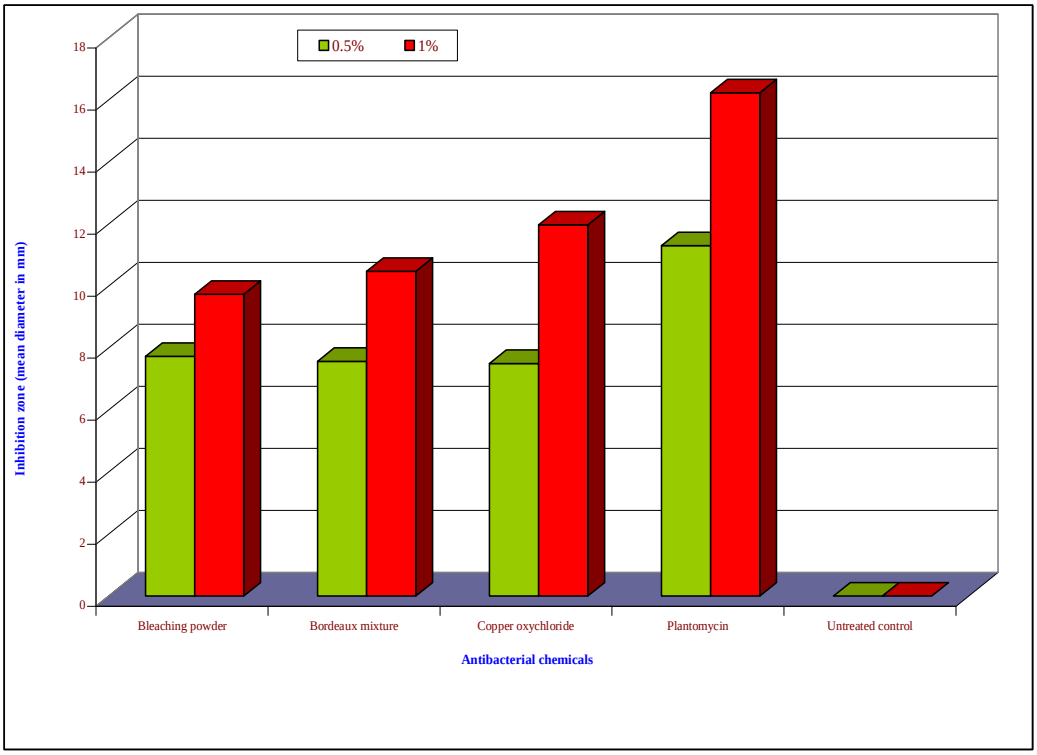
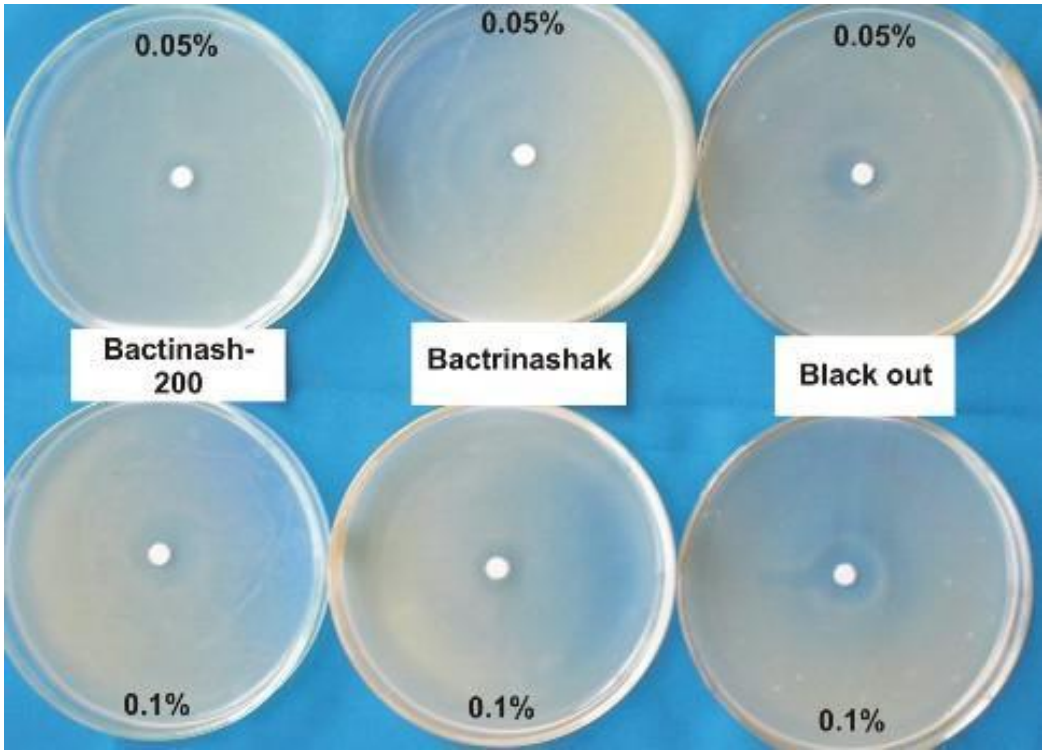


Fig. 24: In vitro evaluation of antibacterial chemicals on the growth of Xanthomonas axonopodis pv. punicae



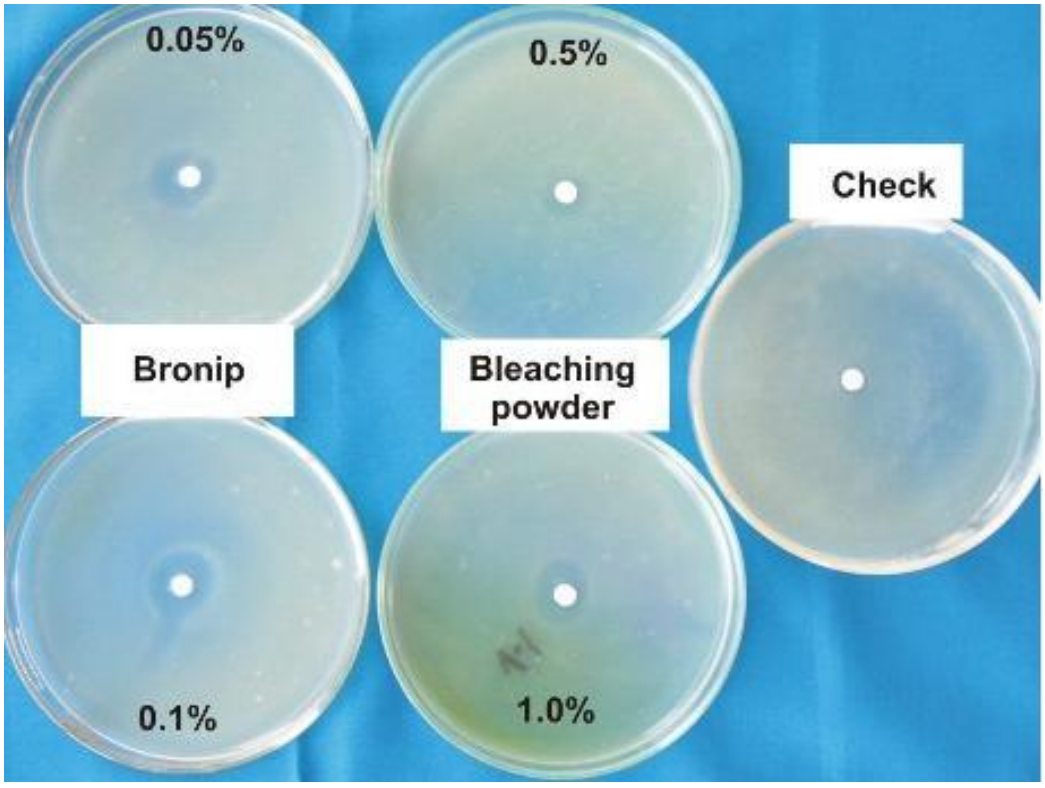


Plate 13: Invitro evaluation of bactericides and antibacterial chemicals on the growth of X. axonopodis pv. punicae

The efficacy between these bactericides was non-significant. The moderate effective treatments were copper oxychloride @ 0.3% (11.97 mm), K-cycline @ 0.1% (11.56), plantomycin @ 0.5% (11.30 mm), Bordeaux mixture @ 1% (10.47 mm) and bactrinashak @ 0.1% (10.10 mm), which were on par in their efficacy with each other. Copper oxychloride @ 0.2% was found least effective with an inhibition zone of 7.50 mm.

4.9.3.2 Field evaluation of bactericides and antibacterial chemicals against bacterial blight of pomegranate

This experiment was carried out in two seasons mrigbahar (June-December, 2007) and ambiabahar (February-August, 2008) to test the field efficacy of different commercially available bactericides/ antibacterial chemicals against the incidence and severity of bacterial blight in pomegranate. The details of the trial are furnished in “Material and Methods” chapter.

4.9.3.2.1 Mrigbahar season trial (2007)

It is observed that, incidence and severity of the bacterial blight before the treatment imposition (Table 26) was absolutely non-significant.

Results after three sprays revealed that, bronip (with 24.16% incidence and 3.64 PDI), bactrinashak (24.11% incidence and 4.51 PDI) and bactinash 200 (27.77% incidence and 4.28 PDI) each at 0.05% concentration used in combination with copper oxychloride (0.2%) were highly effective in reducing the incidence and severity of bacterial blight (Plate 14). The efficacy exhibited between these bactericides was on par and significantly superior over other treatments of the trial except k-cycline (0.05%), which recorded on par efficacy with bactinash-200 and bactinashak in controlling the disease.

Among the antibacterial chemicals, Bordeaux mixture at 1% concentration was significantly superior over bleaching powder at 1% concentration (69.34%) and copper oxychloride at 0.2% concentration (62.84%) in recording less disease incidence of 41.38 per cent. However, its efficacy was on par with the former chemicals in reducing the disease severity (12.57 PDI). Maximum disease incidence and severity of 67.35 pre cent and 21.54 PDI, respectively was recorded in untreated check plot.

Observations after five sprays showed that bronip (0.05%) in combination with COC (0.2%) was significantly effective, which recorded the lowest disease incidence of 12.99 per cent.

The next best effective bactericides were bactrinashak (20.53%), bactinash-200 (23.62%) and K-cycline (27.50%) each at 0.05% concentration used in combination with COC (0.2%). These bactericides were on par in efficacy with each other and were recorded significantly superior efficacy over other treatments of the trial in reducing disease incidence. Among the antibacterial chemicals alone, copper oxychloride (0.2%) recorded significantly less disease incidence of 54.46 per cent and highest disease incidence of 76.43 per cent was noticed in untreated control.

In respect of disease severity after five sprays, it was observed that, all the antibiotics except plantomycin were very effective in reducing the disease severity as these bactericides recorded on par lower disease severity ranged between 3.71 (bactinash-200 + COC) to 4.69 (bactrinashak + COC) per cent disease index.

Antibacterial chemicals *viz.*, bleaching powder @ 1% (23.93 PDI) and copper oxychloride @ 0.2% (24.35 PDI) were non-significant and moderately effective. Bordeaux mixture @ 1% (30.07 PDI) was found least effective in reducing the disease severity.

4.9.3.2.2 Ambiabahar season trial (2008)

Results of the trial revealed that (Table 27), incidence and severity of the disease before the treatment application was non-significant and almost uniform in the plots and significant differences among the treatments were observed after third application of bactericides. Minimum disease incidence of 30.12 per cent was recorded in bronip (0.05%) + copper oxychloride (0.2%) treated plot followed by bactinash-200 (31.54%), bactrinashak (32.81%), K-cycline (34.02%) and streptocycline (36.62%) at similar concentration in combination with COC. The efficacy exhibited by these bactericides was non-significant with each other and differed significantly with other treatments. Plantomycin (0.5%) + copper oxychloride (0.2%) was next best effective chemical. The antibacterial chemicals *viz.*,

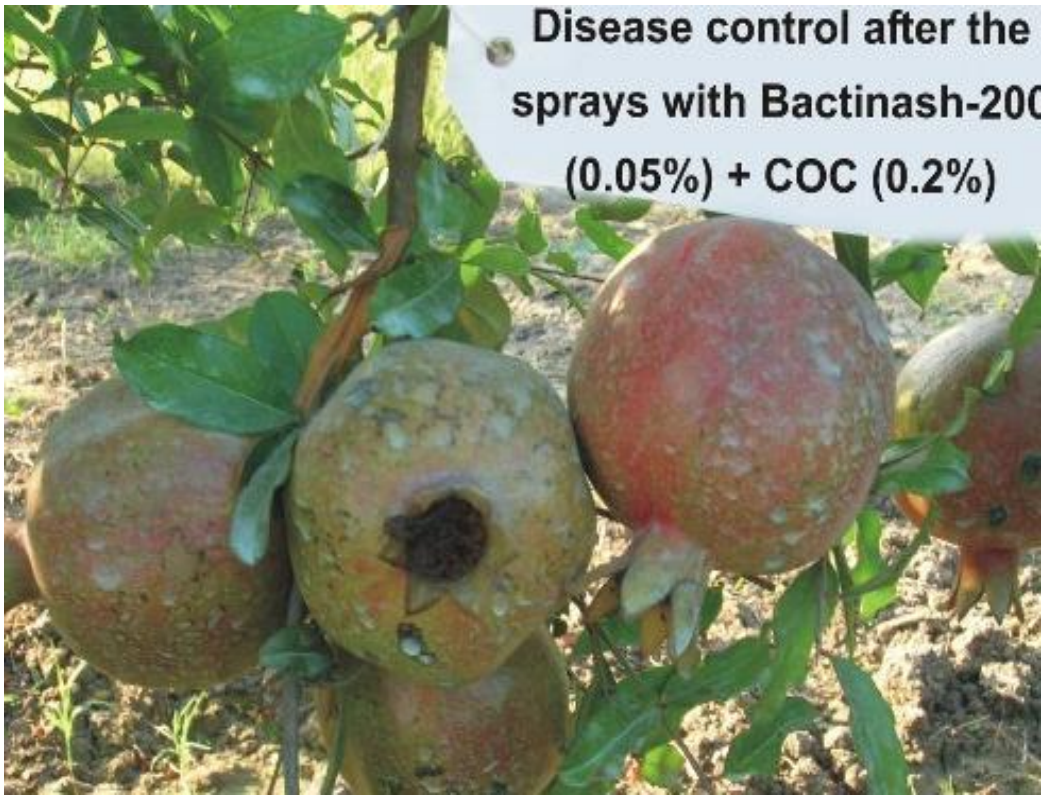
Table 26: Field evaluation of commercially available bactericides and antibacterial chemicals on the incidence and severity of bacterial blight of pomegranate during Mrigbahar (June-December) 2007

Sl. No.	Bactericides / antibacterial chemicals	Concentration (%)	Incidence and severity of bacterial blight on fruits at different spray intervals					
			Before spray		After 3 sprays		After 5 sprays	
			Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index
1.	Bactinash 200 + Copper oxychloride	0.05 0.2	30.69 (33.33)	4.71 (12.37)	27.77 (31.71)	4.28 (11.73)	23.62 (28.99)	3.71 (11.06)
2.	Bactrinashak + Copper oxychloride	0.05 0.2	42.07 (40.42)	6.44 (14.57)	24.11 (29.18)	4.51 (12.02)	20.53 (26.77)	4.69 (12.35)
3.	Bronip + Copper oxychloride	0.05 0.2	29.36 (32.74)	5.73 (13.74)	24.16 (29.39)	3.64 (10.54)	12.99 (21.05)	3.83 (11.02)
4.	K cycline + Copper oxychloride	0.05 0.2	35.04 (36.27)	7.58 (15.97)	31.35 (34.05)	5.76 (13.89)	27.50 (31.62)	4.11 (11.66)
5.	Plantomycin + Copper oxychloride	0.05 0.2	22.52 (28.27)	5.67 (13.62)	36.03 (36.83)	14.24 (21.95)	45.58 (42.52)	17.33 (24.55)
6.	Streptocycline + Copper oxychloride	0.05 0.2	38.21 (38.15)	6.85 (15.13)	35.79 (36.70)	5.26 (13.18)	32.82 (34.92)	4.56 (12.27)
7.	Bleaching powder	1	35.61 (36.61)	5.34 (13.18)	69.34 (56.38)	16.24 (23.59)	61.21 (51.52)	23.93 (29.19)
8.	Bordeux mixture	1	30.73 (33.61)	6.51 (14.63)	41.38 (40.00)	12.57 (20.66)	68.54 (55.91)	30.07 (33.19)
9.	Copper oxychloride	0.2	28.37 (32.15)	5.58 (13.57)	62.84 (52.45)	16.04 (23.38)	54.46 (47.53)	24.35 (29.52)
10.	Untreated control	-	33.53 (35.33)	6.39 (14.55)	67.35 (55.17)	21.54 (27.56)	76.43 (60.99)	44.56 (41.86)
	SEm± CD at 5%		1.89 NS	1.04 NS	1.96 5.84	1.46 4.34	1.78 5.29	1.27 3.77

Figures in parentheses are angular transformed values

Variety : Bhagwa
Time of pruning : II week of July, 2007

Number of sprays : 5
Date of I spray : 24th Sept., 2007
Spray interval : 10 days



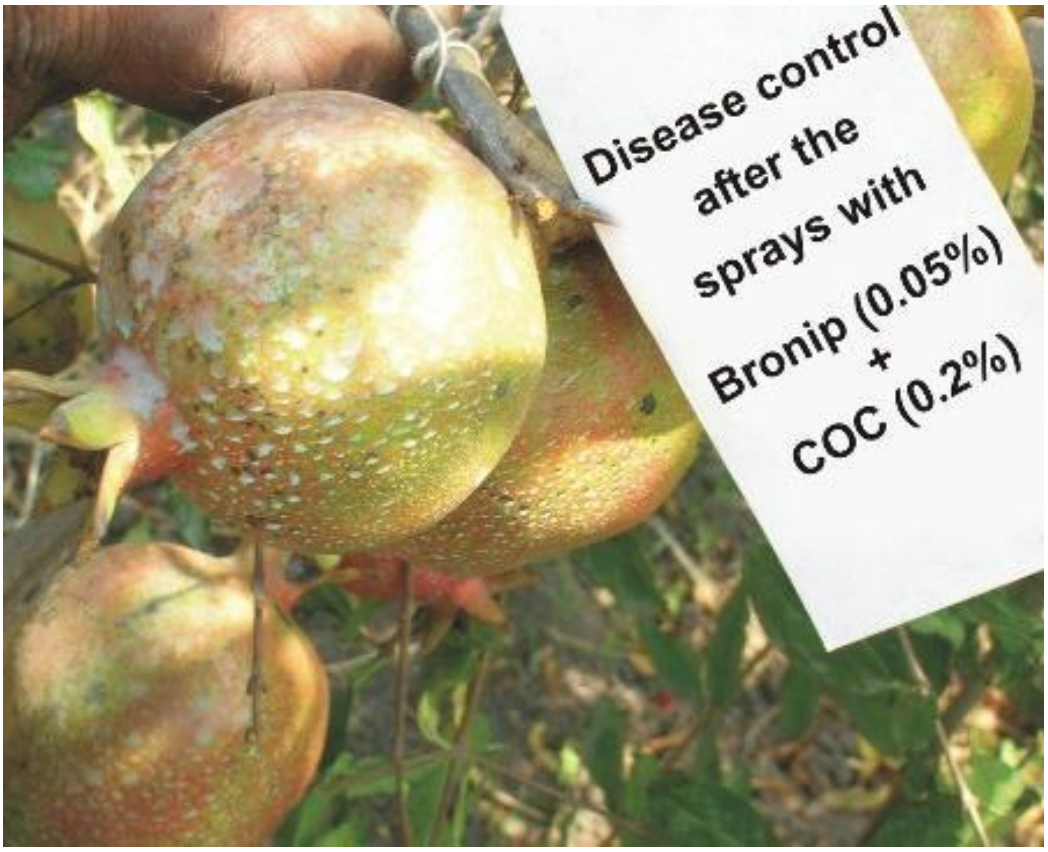


Plate 14: Field efficacy of bactericides in combination with COC against bacterial blight of pomegranate (after five sprays)

Table 27: Field evaluation of commercially available bactericides and antibacterial chemicals on the incidence and severity of bacterial blight of pomegranate during Ambiabahar (February-August) 2008

Sl. No.	Bactericides / antibacterial chemicals	Concentration (%)	Incidence and severity of bacterial blight on fruits at different spray intervals					
			Before spray		After 3 sprays		After 5 sprays	
			Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index
1.	Bactinash 200 + Copper oxychloride	0.05 0.2	41.15 (39.91)	14.35 (22.23)	31.54 (34.12)	12.61 (20.75)	22.90 (28.54)	10.95 (19.18)
2.	Bactrinashak + Copper oxychloride	0.05 0.2	37.65 (37.78)	13.26 (21.26)	32.81 (34.84)	13.50 (21.33)	28.07 (31.88)	11.61 (19.75)
3.	Bronip + Copper oxychloride	0.05 0.2	38.44 (38.22)	17.63 (24.45)	30.12 (33.24)	14.53 (22.21)	21.37 (27.45)	9.74 (17.66)
4.	K cycline + Copper oxychloride	0.05 0.2	37.21 (37.57)	19.00 (25.83)	34.02 (35.66)	17.81 (24.89)	32.20 (34.53)	16.09 (23.44)
5.	Plantomycin + Copper oxychloride	0.05 0.2	37.74 (37.84)	14.28 (22.05)	40.38 (39.43)	16.26 (23.76)	48.70 (44.26)	20.56 (26.91)
6.	Streptocycline + Copper oxychloride	0.05 0.2	39.21 (38.74)	16.30 (23.67)	36.62 (37.23)	13.63 (21.55)	29.44 (32.84)	11.58 (19.75)
7.	Bleaching powder	1	41.59 (40.10)	21.95 (27.84)	45.07 (42.16)	24.17 (29.43)	51.17 (45.69)	27.76 (31.77)
8.	Bordeux mixture	1	42.53 (40.69)	20.51 (26.88)	49.32 (44.62)	26.70 (31.05)	54.22 (47.43)	31.74 (34.22)
9.	Copper oxychloride	0.2	41.17 (39.90)	18.71 (25.60)	53.99 (47.30)	25.74 (30.46)	56.37 (49.67)	28.71 (32.36)
10.	Untreated control	-	39.15 (38.73)	20.42 (26.69)	57.34 (49.24)	31.84 (34.37)	67.98 (55.58)	41.28 (39.95)
	SEm± CD at 5%		2.22 NS	1.96 NS	1.53 4.53	1.64 4.86	1.91 5.68	1.97 5.85

Figures in parentheses are angular transformed values

Variety : Bhagwa
Time of pruning : 10 – 15th March, 2008

Number of sprays : 5
Date of I spray : 12th June., 2008
Spray interval : 10 days

bleaching powder at 1% (45.07%) and Bordeaux mixture at 1% (49.32%) were significantly less effective in reducing the disease incidence and copper oxychloride alone was found least effective with maximum disease incidence of 53.99 per cent and was on par with untreated control (57.34%).

Similarly, disease severity recorded in all the antibiotics (bactericides) with COC treated plot was significantly less and ranged between 12.61 (bactinash 200 + COC) to 17.81 per cent disease index (K cycline + COC) indicating their greater efficacy against the disease. The efficacy of each of these bactericides in combination with COC was on par. The antibacterial chemicals were also found moderately effective by recording lower disease severity ranged between 24.17 (bleaching powder @ 1%) to 26.70 PDI (Bordeaux mixture @ 1%).

Results recorded after the fifth application of respective treatment revealed that, lowest incidence of 21.37 per cent was recorded in bronip (0.05%) + COC (0.2%) treated plot followed by bactinash 200 + COC (22.90%) at the similar concentration. The next best effective treatments were bacterinashak (0.05%) + COC (0.2%) and streptocycline (0.05%) + COC (0.2%), which recorded 28.07 and 29.44 per cent incidence, respectively. The efficacy exhibited by these bactericides was on par with each other, but significantly superior over other treatments trial. Performance of all the antibacterial chemicals in controlling the disease was very poor as these chemicals recorded more disease incidence ranged from 51.17 (bleaching powder @ 1%) to 56.37 PDI (copper oxychloride 0.2%). Highest disease incidence of 67.98 per cent was recorded in untreated check plot.

Looking into the disease severity recorded after fifth spray, it is observed that, lowest disease severity of 9.74 PDI was observed in bronip (0.05%) + COC (0.2%) treated plot followed by bactinash 200 + COC (10.95 PDI), streptocycline + COC (11.58 PDI) and bactrinashak + COC (11.61 PDI) at the similar concentrations. The other bactericides *viz.*, K-cycline (0.05%) + COC (0.2%) and plantomycin (0.5%) + COC (0.2%) were found moderately effective which recorded 16.09 and 20.56 per cent disease index, respectively. The antibacterial chemicals such as bleaching powder @ 1% (27.76 PDI), copper oxychloride @ 0.2% (28.71 PDI) and Bordeaux mixture at 1% (31.74 PDI) were significantly less effective than bactericides. Maximum disease severity *i.e.,* 41.28 PDI was recorded in untreated check plot.

4.9.3.2.3 Pooled performance over the years

Average performance of bactericides and antibacterial chemicals tested over seasons against the incidence and severity of bacterial blight of pomegranate was estimated and presented in Table 28. Data obtained indicated that disease incidence and severity in the experimental plots was non-significant before the imposition of treatments.

Pooled data obtained after third application of treatments revealed that disease incidence was significantly very less in the plots treated with bronip (27.14%) followed by bactrinashak (28.46%), bactinash 200 (29.66%) and K-cycline (32.69%) each used at the concentration of 0.05 per cent and in combination with COC (0.2%) and efficacy exhibited by these bactericides was on par with each other. Streptocycline and plantomycin along with COC (0.2%) were the next best treatments with the disease incidence of 36.21 and 38.21 per cent, respectively.

Among the antibacterial chemicals, Bordeaux mixture (1%) recorded significantly least disease incidence of 45.36 per cent. Maximum disease incidence of 65.98 per cent was recorded in untreated check plot.

Similarly, severity of the disease observed in all the bactricides (antibiotics) treated plots with COC (except plantomycin 0.5% + COC @ 0.2%) were on par with each other. Minimum disease severity of 8.52 PDI was recorded in bactinash-200 + COC followed by 9.01 PDI in bactrinashak @ 0.05% + COC @ 0.2% treated plot. All these bactericides exhibited on par efficacy with each other. The antibacterial chemicals exhibited significantly moderate efficacy against the disease severity in comparison with untreated check.

Average performance of different treatments after the fifth application showed that (Fig. 25) bronip (0.05%) in combination with COC (0.2%) was significantly very effective (Plate 15) than rest of the treatments by recording minimum disease incidence of 17.18 per

Table 28: Field evaluation of commercially available bactericides and antibacterial chemicals against the incidence and severity of bacterial blight of pomegranate over the seasons, Mrigbahar 2007 and Ambiabahar 2008 (Pooled)

Sl. No.	Bactericides / antibacterial chemicals	Concentration (%)	Incidence and severity of bacterial blight on fruits at different spray intervals					
			Before spray		After 3 sprays		After 5 sprays	
			Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index
1.	Bactinash 200 + Copper oxychloride	0.05 0.2	35.92 (36.80)	9.53 (17.96)	29.66 (33.00)	8.52 (16.94)	23.26 (28.82)	7.33 (15.63)
2.	Bactrinashak + Copper oxychloride	0.05 0.2	39.87 (39.14)	9.86 (18.25)	28.46 (32.14)	9.01 (17.36)	24.30 (29.41)	8.15 (16.58)
3.	Bronip + Copper oxychloride	0.05 0.2	33.91 (35.53)	11.66 (19.73)	27.14 (31.38)	9.09 (17.32)	17.18 (24.38)	7.87 (14.68)
4.	K cycline + Copper oxychloride	0.05 0.2	36.13 (36.92)	13.29 (21.38)	32.69 (34.86)	11.79 (20.08)	29.85 (33.10)	10.11 (18.42)
5.	Plantomycin + Copper oxychloride	0.05 0.2	30.14 (33.23)	9.98 (18.35)	38.21 (38.18)	15.25 (22.92)	47.14 (43.38)	18.89 (25.73)
6.	Streptocycline + Copper oxychloride	0.05 0.2	38.72 (38.46)	11.58 (19.79)	36.21 (36.97)	9.45 (17.84)	30.89 (33.89)	8.07 (16.41)
7.	Bleaching powder	1	38.60 (38.42)	13.64 (21.62)	57.21 (49.14)	20.21 (26.69)	56.19 (48.59)	25.84 (30.56)
8.	Bordeux mixture	1	36.63 (37.23)	13.51 (21.58)	45.36 (42.34)	19.64 (26.29)	61.38 (51.61)	30.90 (33.77)
9.	Copper oxychloride	0.2	34.77 (36.10)	12.14 (20.40)	58.42 (49.85)	20.89 (27.15)	55.42 (48.10)	26.54 (30.98)
10.	Untreated control	-	33.17 (35.13)	13.36 (21.42)	65.98 (54.36)	26.69 (31.10)	68.57 (55.92)	42.93 (40.93)
	SEm± CD at 5%		1.60 NS	1.27 NS	1.37 4.06	1.15 3.43	1.32 3.90	1.23 3.66

Figures in parentheses are angular transformed values

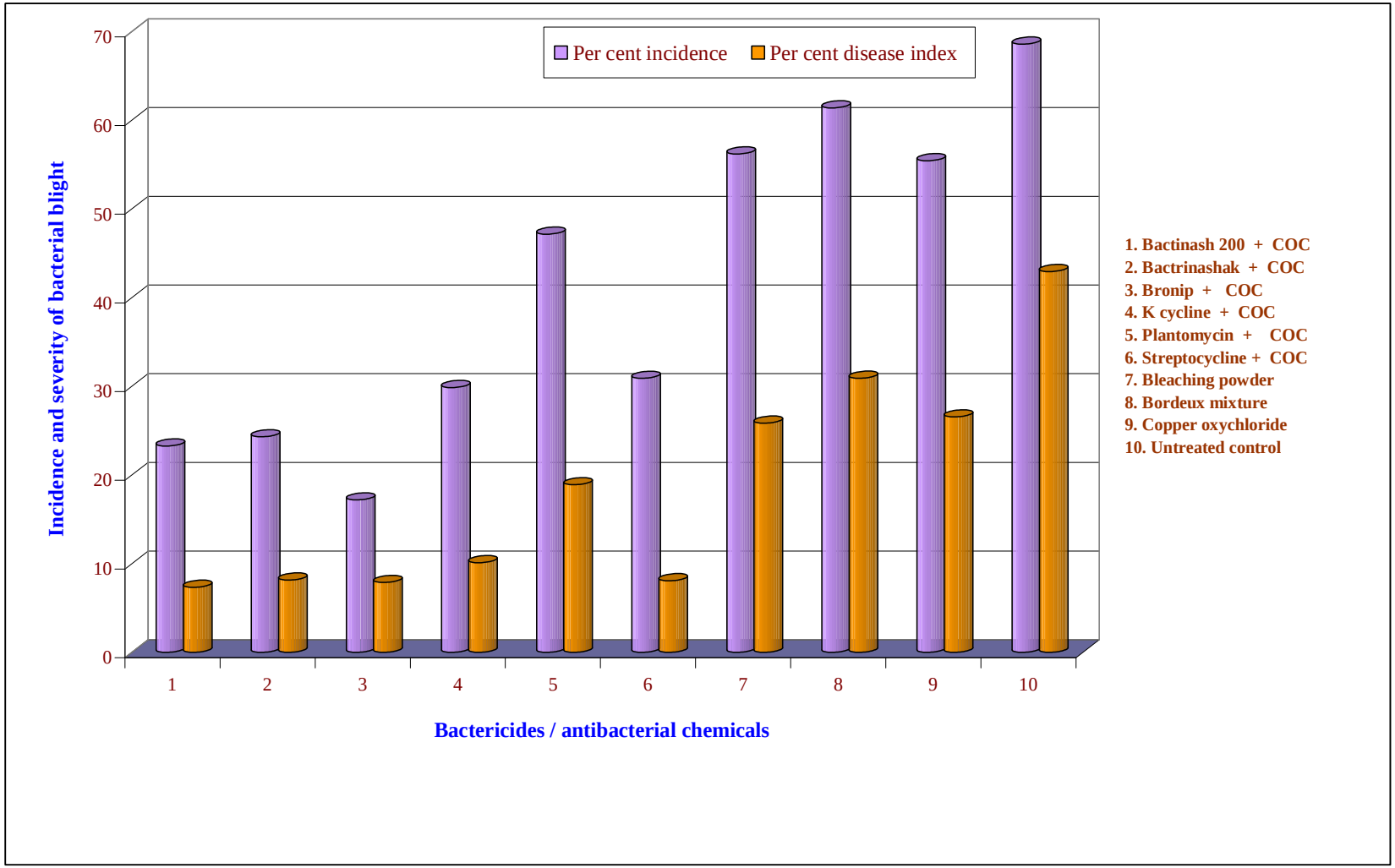


Fig. 25: Field evaluation of commercially available bactericides and antibacterial chemicals against the incidence and severity of bacterial blight of pomegranate over the seasons Mrigbahar 2007 and Ambiabahar 2008 (after five sprays)

Before spray

After first spray

After Third spray

After fifth spray

Plate 15: Stages of bacterial blight control after the spray with bronip (0.05%)+ COC(0.2%) at initial stages of infection

cent. The next best effective treatments were bactinash-200 + COC and bactrinashak + COC at the similar concentrations, which significantly showed lower disease incidence of 23.26 per cent and 24.30 per cent, respectively. K-cycline @ 0.05% with COC 0.2% (29.85%) and streptocycline @ 0.05% with COC 0.2% (30.89%) were found moderately effective against disease incidence. Plantomycin (0.5%) + COC (0.2%) was significantly least effective among all the bactericides (antibiotics) with disease incidence of 47.14 per cent.

Performance of antibacterial chemicals in reducing the disease incidence was very poor with an evidence of >50 per cent disease incidence recorded in the treated plots. In general, disease severity recorded in all the experimental plots was correspondingly less than incidence. However, disease severity was considerably reduced after the fifth application of all the respective bactericides except plantomycin @ 0.5% + COC (0.2%) treated plot. Significantly lower disease severity of 7.33 and 7.87 per cent disease index was recorded by bactinash 200 (0.05%) + COC (0.2%) and bronip (0.05%) + COC (0.2%) respectively followed by streptocycline + COC (8.07 PDI), bacterinashak + COC (8.15 PDI) and K-cycline + COC (10.11 PDI) at the same concentration. All these bactericides exhibited on par efficacy with each other. Among the antibiotics, plantomycin + COC (18.89 PDI) was significantly least effective in reducing the disease severity.

The antibacterial chemicals *viz.*, bleaching powder 1% (25.84 PDI), copper oxychloride @ 0.2% (26.54 PDI) and Bordeaux mixture (30.90 PDI) recorded the moderate disease severity. Significantly maximum disease severity of 42.93 per cent was recorded in untreated check plot.

4.9.3.2 Results on yield

Data on yield parameters revealed that (Table 29), in mrigbahar season, significantly highest fruit yield of 9.72 tonnes per ha was obtained with the treatment by bronip (0.05%) + COC (0.2%) followed by bactrinashak + COC (8.90 t/ha) and bactinash 200 + COC (8.20 t/ha) as an evidence of effective disease control. Significantly lower yield levels ranged between 3.75 tonnes per ha (COC) to 4.25 tonnes per ha (Bordeaux mixture) were recorded with the treatment of antibacterial chemicals. Lowest fruit yield of 1.88 tonnes per ha was recorded in untreated check plot due to maximum disease incidence and severity.

Similarly in ambiabahar season, highest fruit yield of 10.68 tonnes per ha was obtained in bronip + COC treated plot followed by bactinash 200 + COC (9.67 t/ha) and bactrinashak + COC (9.65 t/ha) as influenced by the maximum disease reduction in these plots. The other bactericides *viz.*, streptocycline + COC and K-cycline + COC proved to be moderately effective with on par level yield of 8.34 and 8.25 tonnes per ha, respectively.

The yield obtained in plantomycin + COC treated plot was significantly very low (6.60 t/ha) and was on par with yield of antibacterial treated plots (6.17 – 6.74 t/ha). Significantly lowest yield of 4.71 tonnes per ha was observed in untreated check plot.

Pooled average yield over the seasons due to bacterial blight control by the treatment with bactericides and antibacterial chemicals revealed that, highest average yield of 10.20 tonnes per ha was obtained with bronip (0.05%) + copper oxychloride (0.2%) treatment followed by bactrinashak (0.05%) + COC (9.28 t/ha). The yield levels recorded in these treatments were non-significant with each other (Fig. 26).

The next significant highest yield of 8.94 tonnes per ha was obtained by the treatment of bactinash 200 (0.05%) + COC (0.2%). K-cycline and streptocycline each at 0.05% concentration and in combination with COC (0.2%) significantly recorded moderate yield level of 7.83 and 7.54 tonnes per ha, respectively. The yield obtained in plantomycin (0.05%) + COC (0.2%) treated plot was significantly lower than bactericides and was non-significant with the yield obtained by antibacterial chemical which, ranged between 4.97 (COC @ 0.2%) to 5.43 tonnes per ha (bleaching powder @ 1%). Significantly lowest yield of 3.30 tonnes per ha was observed in untreated check plot.

4.9.4 Evaluation of bioagents

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

The study conducted revealed that, among the five biocontrol agents tried, *Bacillus subtilis* and *Pseudomonas fluorescence* were found significantly superior in inhibiting the growth of the pathogen with an inhibition zone of 16.6 and 16.1 mm diameter (Table 30).

Table 29: Efficacy of different bactericides and antibacterial chemicals on the yield of pomegranate in bacterial blight management trial during 2007 and 2008

Sl. No.	Bactericides / antibacterial chemicals	Concentration (%)	Mrigbahar 2007			Ambiabahar 2008			Pooled over seasons		
			Yield (kg/plant)	Yield (t/ha)	% inc. over control	Yield (kg/plant)	Yield (t/ha)	% inc. over control	Yield (kg/plant)	Yield (t/ha)	% inc. over control
1.	Bactinash 200 + Copper oxychloride	0.05 0.2	16.40	8.20	336.17	19.33	9.67	105.31	17.87	8.94	170.90
2.	Bactrinashak + Copper oxychloride	0.05 0.2	17.80	8.90	373.40	19.30	9.65	104.88	18.55	9.28	181.21
3.	Bronip + Copper oxychloride	0.05 0.2	19.43	9.72	417.02	21.35	10.68	126.75	20.39	10.20	209.09
4.	K cycline + Copper oxychloride	0.05 0.2	14.79	7.39	293.08	16.50	8.25	75.16	15.65	7.83	137.27
5.	Plantomycin + Copper oxychloride	0.05 0.2	9.67	4.83	156.91	13.20	6.60	40.13	11.44	5.72	73.33
6.	Streptocycline + Copper oxychloride	0.05 0.2	13.47	6.73	257.97	16.68	8.34	77.07	15.08	7.54	128.48
7.	Bleaching powder	1	8.23	4.12	119.15	13.48	6.74	43.09	10.86	5.43	64.54
8.	Bordeux mixture	1	8.50	4.25	126.06	12.47	6.24	32.48	10.49	5.25	59.09
9.	Copper oxychloride	0.2	7.50	3.75	99.47	12.35	6.17	30.99	9.92	4.97	50.61
10.	Untreated control	-	3.77	1.88	-	9.42	4.71	-	6.59	3.30	-
	SEm±		0.448	0.224	-	1.302	0.651	-	0.70	0.36	-
	CD at 5%		1.33	0.665	-	3.87	1.934	-	2.08	1.06	-

Figures in parentheses are angular transformed values

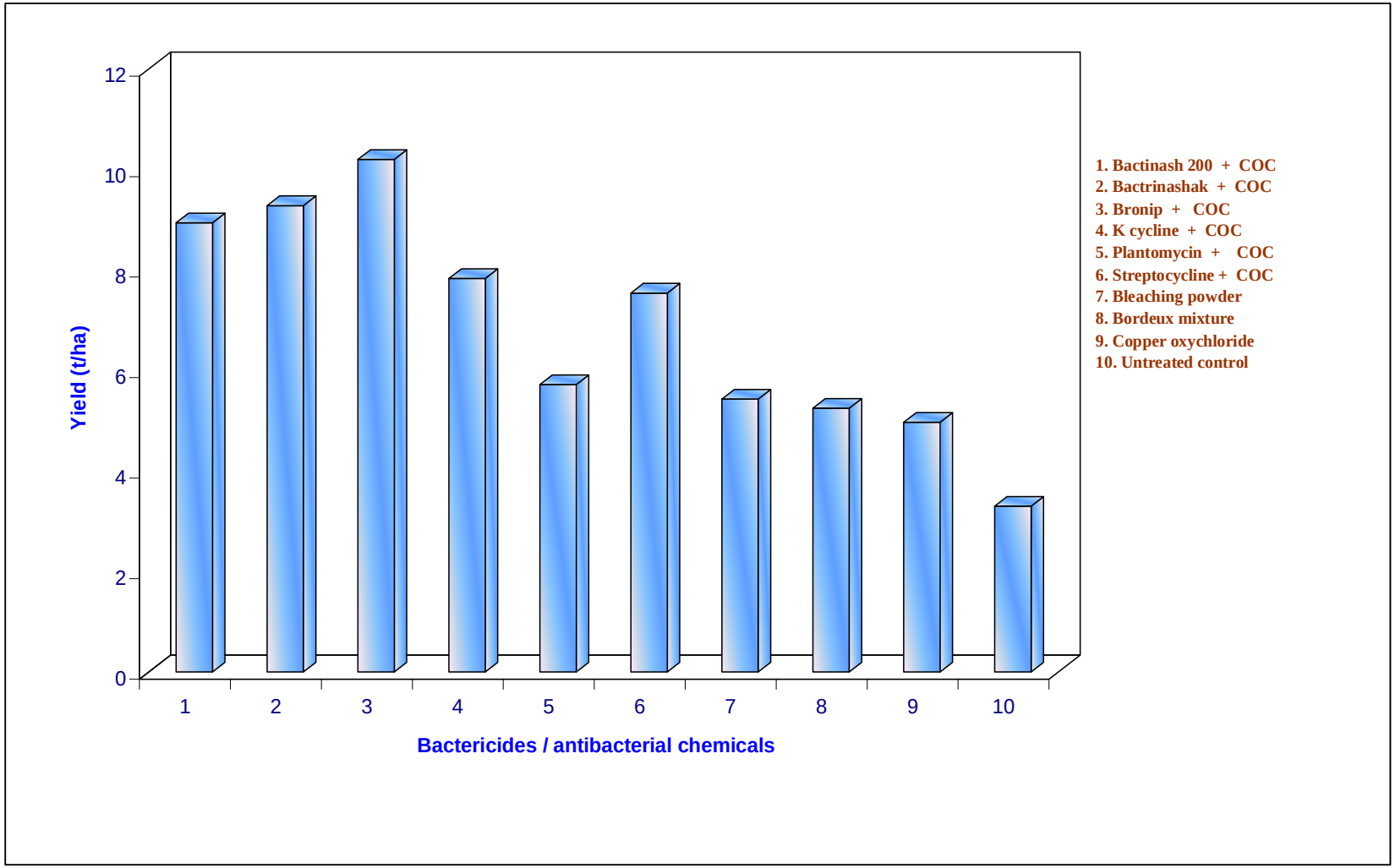


Fig. 26: Efficacy of different bactericides and antibacterial chemicals on the yield of pomegranate in bacterial blight management trial (pooled over the seasons)

Table 30: *In vitro* evaluation of antagonists against *Xanthomonas axonopodis* pv. *punicae*

Sl. No.	Antagonistic organism	Inhibition zone (mean diameter in mm)
1.	*Bacillus subtilis*	16.6 (4.18)*
2.	*Pseudomonas fluorescens*	16.1 (4.13)
3.	*Pseudomonas putida*	0.00 (1.00)
4.	*Trichoderma harzianum*	0.00 (1.00)
5.	*Trichoderma viridae*	0.00 (1.00)
6.	Untreated control	0.00 (1.00)
	SEm±	0.09
	CD at 1%	0.36

* $\sqrt{x+1}$ transformed values

Other biocontrol agents *viz.*, *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas putida* were ineffective as they failed to inhibit the growth of *X. axonopodis* pv. *punicae* (Fig. 27 and Plate 16).

4.9.4.2 Field evaluation of biocontrol agents

The field trial was taken up during Hastbahar cropping season (September-March of 2007-08) to test the field efficacy of different biocontrol agents against bacterial blight of pomegranate. Results are presented in Table 31.

In general, it is observed that, disease pressure was low during the season. However, maximum disease incidence to an extent of 26.15 per cent and maximum severity of 16.80 per cent disease index was noticed in the experimental plots.

Results of the trial indicated that, incidence and severity of the disease before the treatment imposition was non-significant in the experimental plot and was ranged between 17.13 to 20.15 per cent and 3.97 to 5.58 per cent disease index, respectively. Data recorded on disease incidence and severity after third application of the treatments was significant and revealed that combined sprays of *Pseudomonas fluorescence* and *Bacillus subtilis* each at 0.5% concentration was very effective against the incidence (14.88%) and severity (3.33 PDI) of bacterial blight followed by individual sprays of each of these antagonists at 0.5% concentrations. Sprays with *Pseudomonas fluorescence* recorded the lowest incidence of 16.19 per cent followed by *Bacillus subtilis* (16.52%) and remained on par with their combined spray and also with *Pseudomonas putida, Bacillus subtilis* sprayed plot recorded the severity of 3.74 per cent disease index followed by *Pseudomonas fluorescence* (3.90 PDI) and least (3.33 PDI) in the combination of these two bioagents. Further, the efficacy exhibited by these

Fig. 27: *In vitro* evaluation of antagonists against *Xanthomonas axonopodis pv. punicae* under *in vitro* condition

Number	Antagonistic organisms
1	*Bacillus subtilis*
2	*Pseudomonas fluorescens*
3	*Pseudomonas putida*
4	*Trichoderma harzianum*
5	*Trichoderma viridae*
6	Untreated control

Fig. 28: *In vivo* evaluation of antagonists against bacterial blight of pomegranate under field condition during Hastbahar 2007-08 (after five sprays)

Number	Antagonistic organisms
1	*Bacillus subtilis*
2	*Pseudomonas fluorescens*
3	*Pseudomonas fluorescens* + *Bacillus subtilis*
4	*Pseudomonas putida*
5	*Trichoderma harzianum*
6	*Trichoderma viridae*
7	Untreated control

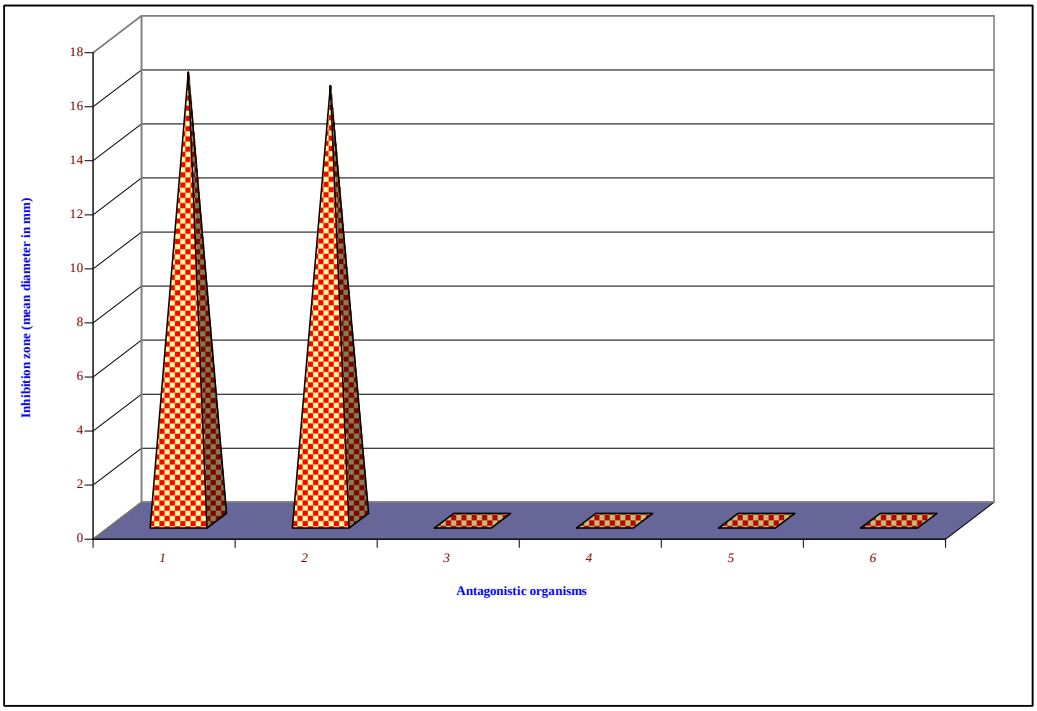


Fig. 27: In vitro evaluation of antagonists against Xanthomonas axonopodis pv. punicae under in vitro condition

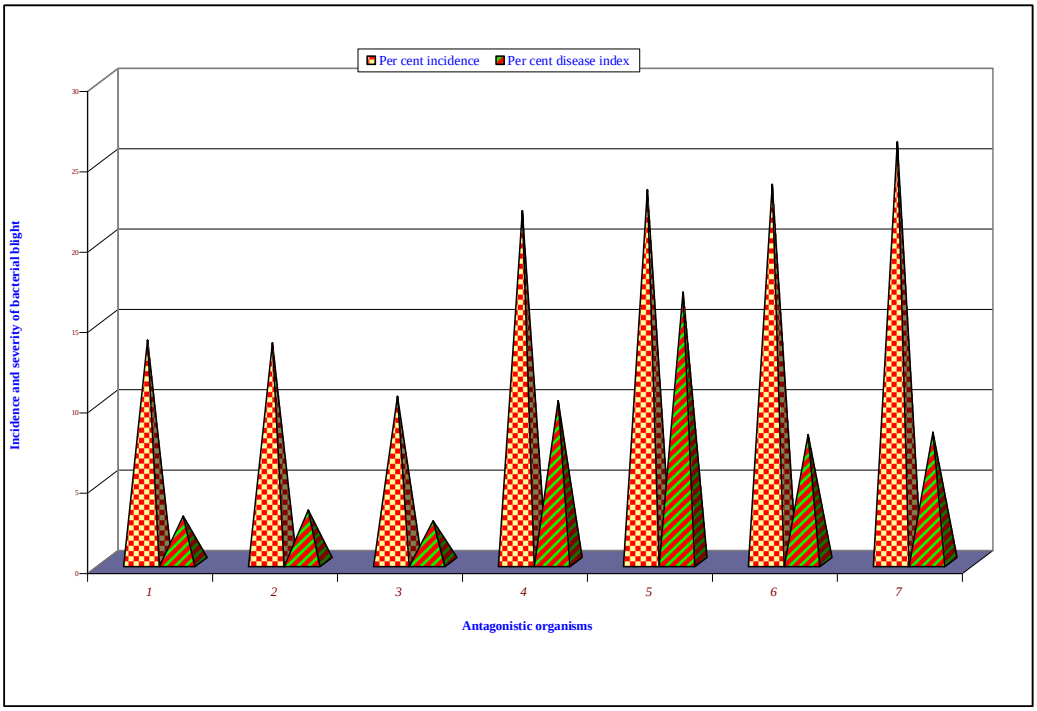
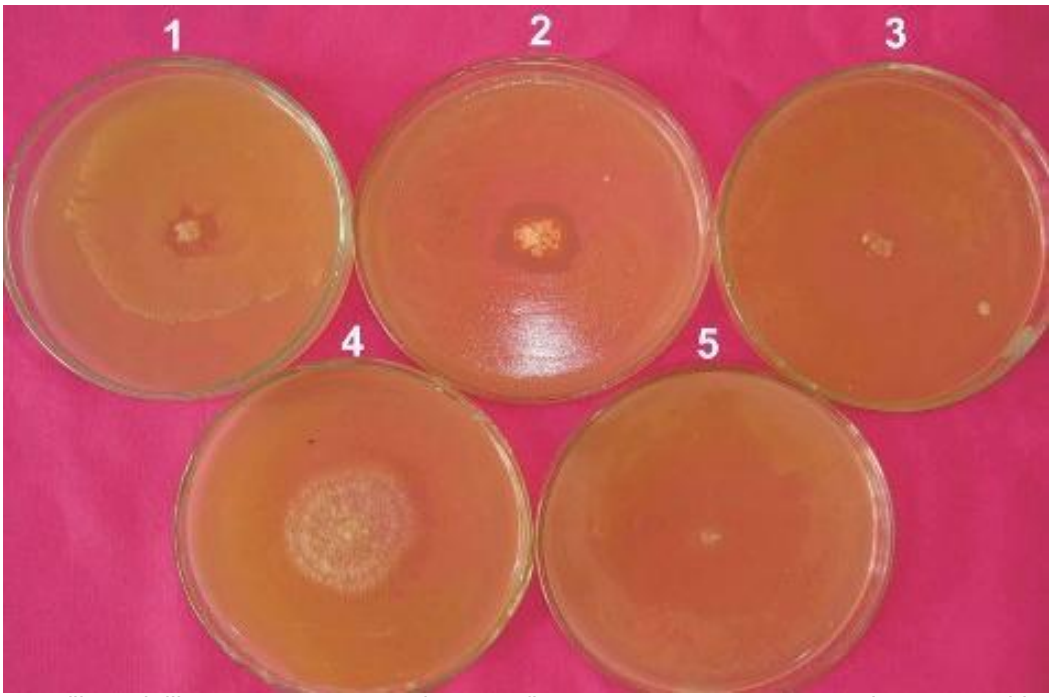
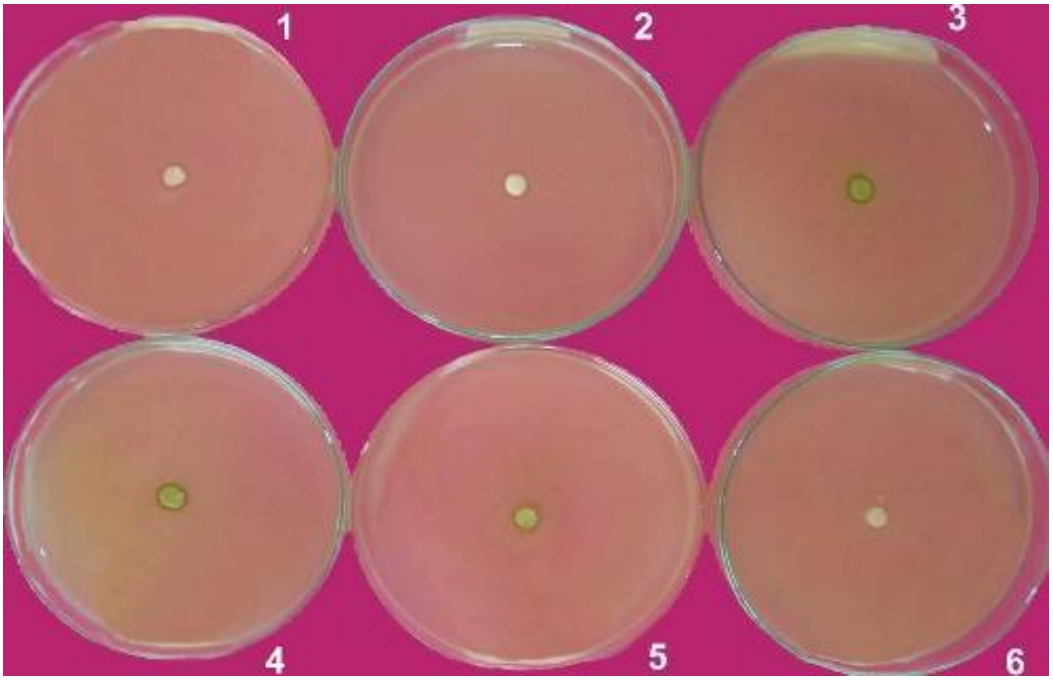


Fig. 28: In vivo evaluation of antagonists against bacterial blight of pomegranate under field condition during Hastbahar 2007-08 (after five sprays)



1.Bacillus subtilis 2.Pseudomonas fluorescens 3.Pseudomonas putida
4.Trichoderma harzianum 5. Untreated check

Plate 16: In vitro evaluation of bioagents against X. axonopodis pv.punicae



1. Garlic bulb extract 2. Onion bulb extract 3. Parthenium leaf extract
4. Lantana leaf extract 4. Ocimum leaf extract 4. Untreated control

Plate 17: In vitro evaluation of botanicals against X. axonopodis pv. punicae

two antagonists irrespective of either individual or combined applications remained non-significant with one another and significantly superior over other antagonists in the trial.

The fungal biocontrol agents *viz.*, *Trichoderma viride* and *Trichoderma harzianum* were less effective with more disease incidence (21.50 and 21.86%) and severity (7.12 PDI and 6.04 PDI), respectively.

Similar trend in respect of efficacy of biocontrol agents was observed after the fifth application of treatments. It was observed that, sprays with *Pseudomonas fluorescence* (0.5%) in combination with *Bacillus subtilis* significantly reduced the incidence (10.32%) and severity (2.56 PDI) of bacterial blight (Fig. 28). The next best effective treatments were individual application of same biocontrol agents, which were on par with each other. Application of *Pseudomonas fluorescence* (0.5%) recorded significantly least incidence 13.64 per cent and severity of 3.24 PDI whereas *Bacillus subtilis* (0.5%) recorded incidence and severity of 13.82 per cent and 2.86 per cent disease index, respectively.

The other biocontrol agents such as *Trichoderma viride* and *Trichoderma harzianum* were found ineffective in reducing the disease as these treatments resulted in more disease incidence (23.50% and 23.17%) and severity (7.93 PDI and 16.80 PDI), respectively. Highest disease incidence of 26.15 per cent was recorded in untreated check plot.

4.9.4.3 Yield obtained in biological control trial

In consequence to the effective disease control in *Pseudomonas fluorescence* (0.5%) + *Bacillus subtilis* (0.5%) treated plot, highest and significant yield of 10.82 tonnes per ha (Table 31) was recorded by the former treatment. The next highest on par yield level of 10.30 and 9.62 tonnes per ha were recorded by individual treatment of *Bacillus subtilis* (0.5%) and *Pseudomonas fluorescence*, respectively. Significantly lower yield was obtained by *Pseudomonas putida* (5.43 t/ha) and *Trichoderma harzianum* (5.25 t/ha) each at 0.5% concentration. Lowest yield of 4.38 tonnes per ha was recorded in untreated check plot.

4.9.5 Effect of botanicals

4.9.5.1 *In vitro* evaluation of plant extracts

Plant extracts are known for safe, ecofriendly and cost effective means of managing the crop diseases effectively. The present study was conducted with nine different plant extracts screened at 5% and 10% concentrations against the growth of *X. axonopodis* pv. *punicae* under *in vitro* conditions. The results obtained are presented in Table 32.

Among the different plant extracts under evaluation, garlic bulb extract was found very effective (Plate 17) in inhibiting the maximum growth of the pathogen by 8.11 mm diameter followed by parthenium leaf extract (7.56 mm), onion bulb extract (7.24 mm) and lantana leaf extract (7.11 mm). These treatments were non-significant with each other in respect of their efficacy and were significantly superior over all other plant extracts screened in the trial. Ocimum leaf extract was found moderately effective with an inhibition zone of 5.50 mm diameter (Fig. 29). The other plant extracts *viz.*, turmeric rhizome extract, zinger rhizome extract, clerodendron leaf extract and neem seed extract were observed completely ineffective with zero efficacy. Between the concentrations, significant difference was observed and higher concentration (10%) was superior to lower concentration (5%).

Interaction effect between the plant extracts and concentrations revealed that, garlic extract at 10% concentration was significantly superior over al other treatments with highest inhibition zone of 9.33 mm (diameter) followed by parthenium leaf extract (8.56 mm), lantana leaf extract (8.00 mm) and onion bulb extract (7.89 mm) each at 10% concentration. The next best effective extracts were garlic bulb extract (6.89 mm) and parthenium leaf extract (6.55 mm) each at 5% concentration. Ocimum leaf extract (5%) was found least effective with an inhibition zone of 4.67 mm (diameter) among the effective plant extracts.

4.9.5.2 Field evaluation of botanicals

A total of nine different plant extracts each @ 10% concentration were subjected for field evaluation aimed at managing the bacterial blight of pomegranate. It was observed that incidence and severity of the disease before the treatment application was non-significant in the experimental plot (Table 33).

Table 31: *In vivo* evaluation of antagonists against bacterial blight of pomegranate under field condition during Hastbahar 2007-08

Sl. No.	Antagonistic organisms	Conc. (%)	Incidence and severity of bacterial blight on fruits						Yield		% increase over untreated control
			Before application		After 3rd application		After 5th application				
			Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	kg/plant	Tones/ ha	
1.	*Bacillus subtilis*	0.5	20.15 (26.67)	5.28 (13.20)	16.52 (23.97)	3.74 (11.08)	13.82 (21.75)	2.86 (9.70)	20.59	10.30	135.16
2.	*Pseudomonas fluorescens*	0.5	18.44 (25.38)	4.20 (11.79)	16.19 (23.59)	3.96 (11.43)	13.64 (21.49)	3.24 (10.25)	19.24	9.62	119.63
3.	*Pseudomonas fluorescens* + *Bacillus subtilis*	0.5 0.5	17.37 (24.60)	3.97 (11.44)	14.88 (22.64)	3.33 (10.52)	10.32 (18.60)	2.56 (9.16)	21.62	10.82	147.03
4.	*Pseudomonas putida*	0.5	18.69 (25.55)	4.58 (12.31)	19.05 (25.83)	7.54 (15.93)	21.86 (27.84)	10.04 (18.46)	10.84	5.43	23.97
5.	*Trichoderma harzianum*	0.5	17.13 (24.43)	4.17 (11.71)	21.86 (27.82)	6.04 (14.20)	23.17 (28.74)	16.80 (15.06)	10.49	5.25	19.86
6.	*Trichoderma viridae*	0.5	19.80 (26.40)	5.58 (13.54)	21.50 (27.57)	7.12 (15.36)	23.50 (26.93)	7.93 (16.26)	12.49	6.25	42.69
7.	Untreated control	-	19.14 (25.92)	4.34 (11.99)	23.72 (29.10)	6.98 (15.25)	26.15 (30.73)	8.08 (16.47)	8.75	4.38	-
	SEm+		0.89	0.80	1.24	0.71	1.44	0.69	1.08	0.54	
	CD at 5%		NS	NS	3.67	2.11	4.28	2.04	3.20	1.60	

Figures in parentheses are angular transformed values

Variety : Bhagwa
Date of pruning : 8 – 10th Sept., 2007
Number of sprays : 5
Date of I spray : 19th Nov., 2007
Spray interval : 10 days

Table 32: Bioefficacy of different plant extracts against *Xanthomonas axonopodis* pv. *punicae* under *in vitro* conditions

Sl. No.	Plant extracts	Inhibition zone (mean diameter in mm)		
		5%	10%	Mean
1.	Clerodendron leaf extract	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)
2.	Garlic bulb extract	6.89 (2.80)	9.33 (3.21)	8.11 (3.01)
3.	Lantana leaf extract	6.22 (2.68)	8.00 (3.00)	7.11 (2.84)
4.	Neem seed extract	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
5.	Ocimum leaf extract	4.67 (2.38)	6.33 (2.71)	5.50 (2.55)
6.	Onion bulb extract	6.58 (2.75)	7.89 (2.98)	7.24 (2.87)
7.	Parthenium leaf extract	6.55 (2.74)	8.56 (3.09)	7.56 (2.92)
8.	Turmeric rhizome extract	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
9.	Zinger rhizome extract	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
10.	Untreated control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	Mean	3.09 (1.84)	4.01 (1.99)	3.55 (1.92)

	Source	SEm	CD at 1%
	Plant extracts (P)	0.05	0.20
	Concentration (C)	0.02	0.09
	Interaction (P × C)	0.07	0.28

* $\sqrt{x+1}$ transformed values

Results obtained after third application of treatments indicated that, garlic and onion bulb extracts were significantly very effective in controlling the disease as the lowest incidence and severity of the disease was observed in the treated plots of these extracts. Garlic bulb extract recorded least incidence of 21.47 per cent with a minimum disease severity of 6.35 PDI followed by onion bulb extract with incidence and severity of 23.90 per cent and 6.85 PDI, respectively. The other botanical extracts *viz.*, parthenium, neem seed, zinger, lantana, clerodendron, turmeric and ocimum leaf extracts were proved ineffective after the three sprays of these extracts.

Results on efficacy of these extracts after the fifth application was not much significant as there was negligible reduction in the incidence and severity of the disease in

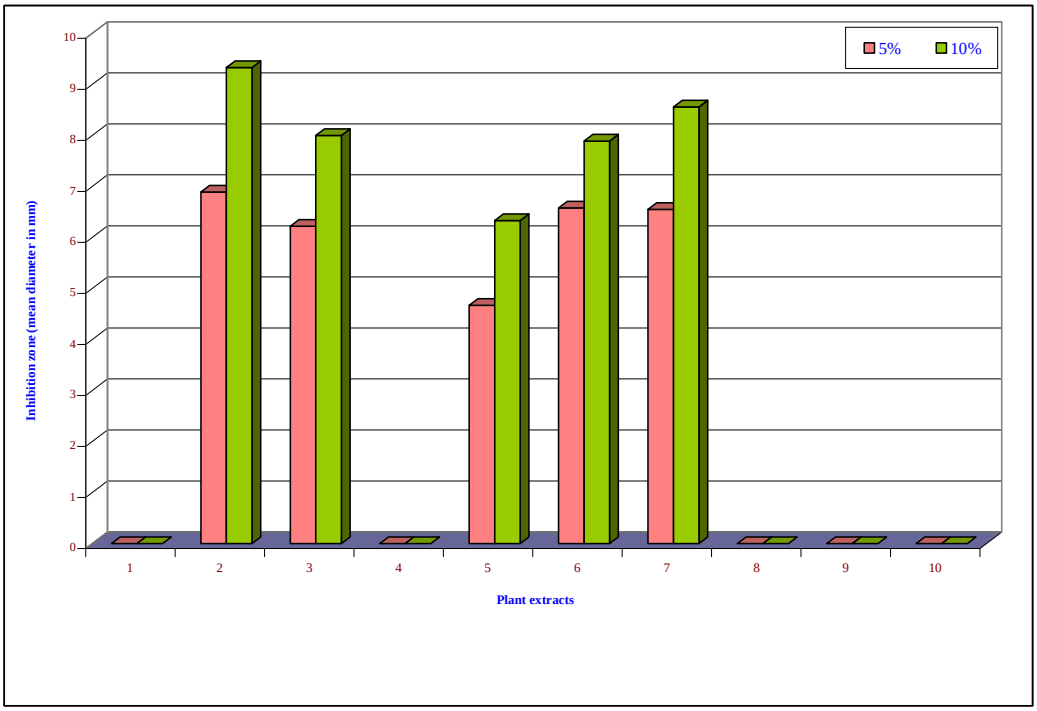


Fig. 29: Bioefficacy of different plant extracts against the growth of Xanthomonas axonopodis pv. punicae under in vitro conditions

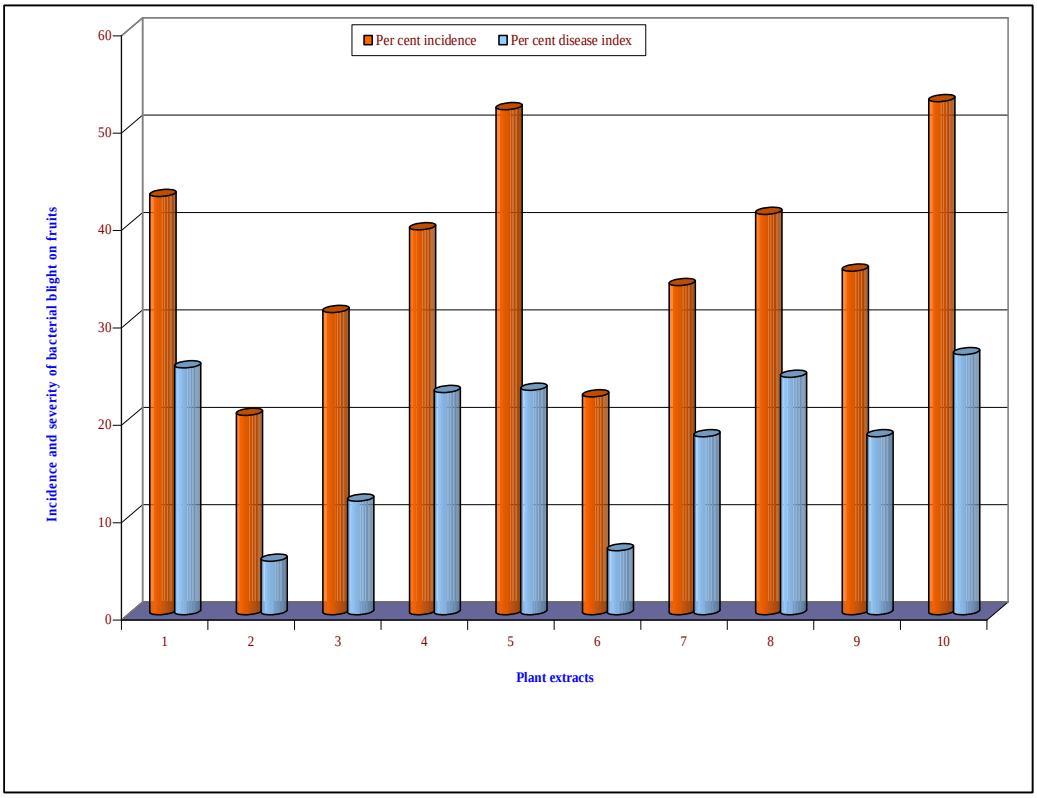


Fig. 30: In vivo evaluation of botanicals against bacterial blight of pomegranate (after fifth application)

Table 33: *In vivo* evaluation of botanicals against bacterial blight of pomegranate

Sl. No.	Plant extract	Conc. (%)	Incidence and severity of bacterial blight on fruits						Yield		% increase over untreated control
			Before application		After 3rd application		After 5th application				
			Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	kg/plant	tones per ha	
1.	Clerodendron leaf extract	10	24.67 (29.77)	7.88 (16.26)	34.70 (36.04)	12.88 (21.04)	42.93 (40.93)	25.34 (30.24)	9.60	4.80	-
2.	Garlic bulb extract	10	24.75 (29.80)	8.84 (17.23)	21.47 (27.58)	6.35 (14.44)	20.47 (26.87)	5.49 (13.40)	18.14	9.07	87.39
3.	Lantana leaf extract	10	23.41 (28.89)	7.54 (15.95)	29.19 (32.66)	9.32 (17.66)	31.03 (33.83)	11.66 (19.95)	16.02	8.01	65.49
4.	Neem seed kernel extract	10	20.95 (27.20)	8.33 (16.77)	27.24 (31.43)	13.91 (21.88)	39.52 (38.87)	22.81 (28.50)	13.48	6.74	39.26
5.	Ocimum leaf extract	10	22.74 (28.43)	7.16 (15.53)	30.92 (33.72)	9.54 (17.93)	51.85 (46.09)	23.04 (28.64)	10.13	5.07	4.75
6.	Onion bulb extract	10	27.47 (31.55)	8.26 (16.64)	23.90 (29.20)	6.85 (15.10)	22.36 (28.13)	6.57 (14.77)	17.62	8.81	82.02
7.	Parthenium leaf extract	10	23.15 (28.70)	8.02 (16.42)	27.67 (31.66)	12.12 (20.33)	33.80 (35.53)	18.26 (25.20)	14.83	7.44	53.72
8.	Turmeric rhizome extract	10	24.82 (29.81)	8.29 (16.68)	29.30 (32.76)	13.56 (21.53)	41.11 (39.86)	24.38 (29.56)	11.98	5.99	23.76
9.	Zinger rhizome extract	10	21.38 (27.50)	5.89 (14.05)	28.23 (32.03)	11.56 (19.83)	35.28 (36.37)	18.27 (25.24)	13.28	6.64	37.19
10.	Untreated control	- -	25.94 (30.56)	9.07 (17.44)	39.64 (38.99)	14.92 (22.50)	52.68 (46.55)	26.70 (31.02)	9.68	4.84	-
	SEm±		1.41	0.80	1.52	1.19	1.71	1.43	0.94	0.47	-
	CD at 5%		NS	NS	4.50	3.54	5.08	4.26	2.78	1.39	-

Figures in parentheses are angular transformed values

Variety : Bhagwa
Date of pruning : 10 - 15th March, 2008
Number of sprays : 5
Date of I spray : 07-06-2008
Spray interval : 10 days

Table 34: Effect of micronutrients on the incidence and severity of bacterial blight of pomegranate

Sl. No.	Micronutrients	Method of application and dosage	Incidence and severity of bacterial blight on fruits				Yield		Per cent increase in yield over untreated control
			After soil application and two sprays		After soil application and four sprays				
			Per cent incidence	Per cent disease index	Per cent incidence	Per cent disease index	kg/plant	tones/ha	
1.	Calcium sulphate ($CaSO_4$)	Soil application (100 g/pl) + foliar sprays (1%)	30.70 (33.59)	12.25 (20.40)	42.27 (40.56)	18.04 (25.06)	13.63	6.82	75.32
2.	Ferrous sulphate ($FeSO_4$)	------ do -----	29.22 (32.69)	13.18 (21.25)	44.48 (41.86)	20.64 (26.95)	11.71	5.86	50.64
3.	Magnesium sulphate ($MgSO_4$)	------ do -----	42.24 (40.53)	11.89 (20.10)	59.30 (50.37)	18.22 (25.27)	8.43	4.22	8.48
4.	Multinutrients (Zn, Mg, B, Fe, Cu, Mn, Mo & S)	------ do -----	26.65 (30.99)	9.86 (18.23)	34.02 (35.64)	13.22 (21.28)	15.99	8.00	105.66
5.	Solu B (Boran)	------ do -----	37.87 (37.96)*	15.80 (23.34)	60.13 (50.89)	34.96 (36.17)	7.33	3.67	-
6.	Zinc sulphate ($ZnSO_4$)	------ do -----	45.84 (42.62)	30.70 (33.62)	62.61 (52.35)	48.01 (43.85)	6.54	3.27	-
7.	Untreated control	-	47.57 (43.62)	22.26 (28.11)	57.49 (49.31)	41.17 (39.92)	7.78	3.89	-
	SEm±		1.57	1.07	1.50	1.43	0.89	0.49	
	CD at 5%		4.65	3.17	4.48	4.24	2.54	1.48	

Figures in parentheses are angular transformed values

Variety : Bhagwa
Cropping season : Ambiabahar (2008-09)
Date of pruning : 10 – 15th March, 2008

Time of micronutrient application
Soil application : One month after pruning
Foliar application (I spray) : At fruiting stage
No. of sprays : 4
Spray interval : 10 days

garlic and onion extracts treated plots. Garlic bulb extract recorded minimum incidence of 20.47 per cent and severity of 5.49 PDI, while onion bulb extract recorded 22.36 per cent incidence and 6.57 PDI (severity). Although, the efficacy exhibited by these two treatments was on par with each other and significantly differed over other treatments.

Among the rest of the botanicals, lantana and parthenium leaf extracts significantly recorded the moderate incidence and severity of the disease. Lantana leaf extract recorded 31.03 per cent incidence and 11.66 PDI (severity) followed by parthenium leaf extract with incidence and severity of 33.80 per cent and 18.26 PDI, respectively. Significantly more disease incidence ranged between 40 to 50 per cent and severity ranged between 23 to 25 per cent was recorded by turmeric rhizome extract, clerodendron leaf extract and ocimum leaf extract. Among all the botanicals, ocimum leaf extract was least effective with more disease incidence (51.85%) and severity (23.04 PDI). Highest disease incidence of 52.68 per cent and severity of 26.70 PDI was recorded in untreated check plot (Fig. 30).

4.9.5.3 Yield as influenced by plant extracts

Yield obtained in different plant extract treated plots indicated that, highest and significant yield of 9.07 tonnes per ha was recorded in garlic bulb extract treated plot (Table 33). The next higher on par yield levels were obtained in onion bulb extract (8.81 t/ha), lantana leaf extract (8.01 t/ha) and parthenium leaf extract (7.44 t/ha) treated plots, which significantly differed with the turmeric rhizome extract (5.99 t/ha) and ocimum leaf extract (5.07 t/ha). Celrodendron leaf extract recorded the lowest yield of 4.80 tonnes per ha, which was on par with the yield of untreated control (4.84 t/ha) plot.

4.9.6 Role of micronutrients on the incidence and severity of bacterial blight in pomegranate

The field study was conducted to know the significance of application of micronutrients such as zinc, boron, magnesium, calcium, iron and multinutrients in reducing the incidence and severity of bacterial blight in pomegranate and results are presented in Table 34.

Disease situation analysed after the two sprays (with soil application) of individual treatment showed the minimum incidence (26.65%) and severity (9.86 PDI) of the disease in multinutrients treated plot followed by the treatments with iron and calcium, which recorded on par incidence (29.22% and 30.70%) and severity (13.18 PDI and 12.25 PDI), respectively.

The efficacy exhibited by the former treatments were significantly superior over other treatments in the trial. The next best treatment was application of boron, which recorded non-significant incidence (37.87%) and severity (15.80 PDI) with calcium sulphate application.

Application of zinc and magnesium was found significantly ineffective with more disease incidence of 45.84 and 42.24 per cent, respectively and their efficacy was on par with untreated check plot in respect of incidence, but disease severity was significantly low in magnesium (11.89 PDI) treated plot.

Results obtained after four sprays of micronutrients revealed the significant minimum incidence (34.02%) and severity (13.22 PDI) in multinutrient treated plot followed by calcium sulphate which recorded 42.27 per cent incidence and 18.04 PDI (severity). The other micronutrients such as zinc, boron and magnesium were found significantly ineffective as these treatments recorded more disease incidence than untreated check (57.49%). However, magnesium treated plot recorded significantly least disease severity of 18.22 per cent disease index.

In general yield recorded by any one of the treated plot was low, because of ineffective disease control. However, among the treatments, highest yield of 8.00 tonnes per ha (Table 34) was recorded in multinutrient treated plot followed by calcium sulphate (6.82 t/ha) and remained on par with each other and significantly superior over other treatments of the trial. Significantly lower yields, ranged between 3.27 (zinc) to 4.22 tonnes per ha (magnesium) were obtained by zinc, boron and magnesium. Yield obtained in these treatments were on par with the yield of untreated check (3.89 t/ha).

4.9.7 Evaluation of integrated disease management strategy

Based on the results obtained on various aspects of bacterial blight management in pomegranate, integrated disease management module was formulated with feasible fitment of

a. Crop after pruning

b. Collection and burning of infected debris

c. Clean sanitation of the orchard

d. Application of organic manure to the soil

e. Application of bleaching powder around the basin of the plant

f.Stem pasting with bronip(0.05%)+COC(0.2%)

Plate 18: Practices followed in IDM

different methods of disease reduction, such as field sanitation, stem treatment with bactericides, chemical and biological protection and application of micronutrients (Plate 18). The formulated module was evaluated in two seasons, mrigbahar and hastbahar of 2008 on varieties Bhagwa and Ganesha, respectively with farmer method of disease control as a check treatment.

Results of the study indicated that during mrigbahar on Bhagwa variety, the plot with IDM module recorded the lowest disease incidence (21.78%) and severity (12.35 PDI) in comparison with farmer method of disease control, which recorded incidence and severity of 45.52 per cent and 23.79 PDI (Table 35a), respectively. Similar trend was observed during hastbahar cropping season on Ganesha variety, wherein lowest incidence of 9.46 per cent and severity of 4.58 PDI was recorded by the treatment of integrated management as against the higher disease incidence (22.37%) and severity (10.51 PDI) in farmers practice.

Average intensity of the disease over the seasons indicated that, lowest incidence of 15.62 per cent and severity of 8.47 PDI (Table 35b) was observed with the integrated strategy. Comparatively higher disease incidence (33.94%) and severity (17.15 PDI) was noticed in farmers method of disease control.

Yield attributing parameters in treated plots revealed that under integrated management (Plate 19) on an average 105.58 (Table 35c) number of marketable fruits were harvested per plant with an average fruit size of 190 grams contributing towards higher yield of 10.03 tonnes per ha. Comparatively less number of fruits (71.37) accounting for lower yield of 6.78 tonnes per ha were observed in the treatment of farmers method.

Looking into the economics worked out by taking yield parameter into consideration, highest net profit of Rs. 1,91,318.75 was realized with benefit:cost ratio of 21.6:1 with the adoption of IDM module for bacterial blight management in pomegranate. On the other side, less net profit of Rs. 125200.00 with less benefit cost ratio of 13.03:1 was observed in farmers method of disease control.

Table 35a: Evaluation of integrated disease management module

Treatments	Incidence and severity of bacterial blight on fruit					
	Season : Mrigbahar, 2008, var. Bhagwa			Season : Hastbahar, 2008, var. Ganesha		
	Disease incidence (%)	Per cent disease index	Yield (t/ha)	Disease incidence (%)	Per cent disease index	Yield (t/ha)
T_1 – Integrated strategy	21.78	12.35	11.46	9.46	4.58	8.59
T_2 – Farmers practice	45.52	23.79	7.64	22.37	10.51	5.92

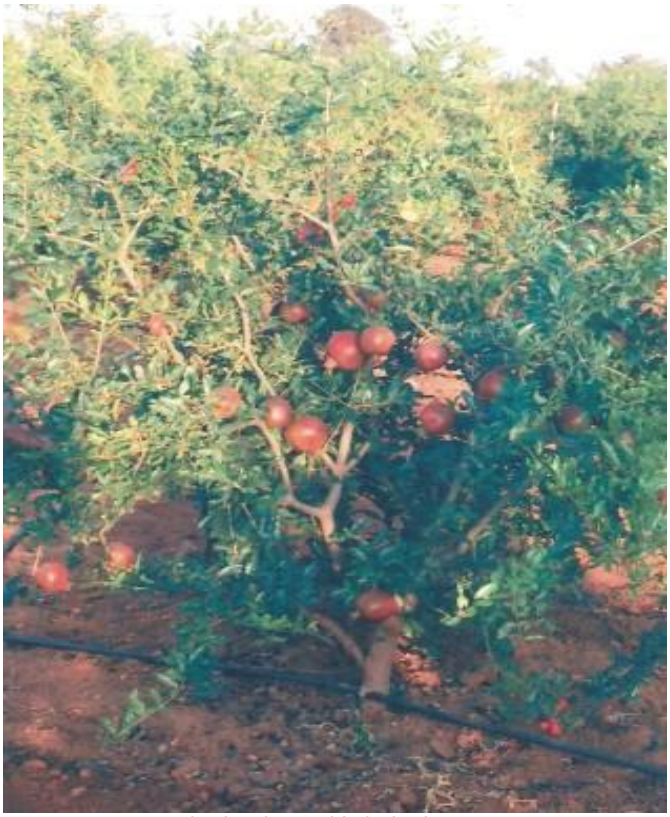
Table 35b: Mean incidence and severity of the disease over seasons

Treatments	Disease incidence (%)			Per cent disease index			Yield (t/ha)		
	Mrig bahar	Hast bahar	Average	Mrig bahar	Hast bahar	Average	Mrig bahar	Hast bahar	Average
T_1 – Integrated strategy	21.78	9.46	15.62	12.35	4.58	8.47	11.46	8.59	10.03
T_2 – Farmers practice	45.52	22.37	33.94	23.79	10.51	17.15	7.64	5.92	6.78

Table 35c: Economics of bacterial blight management in pomegranate by IDM approach

Treatment details	Details of expenditure (Rs./ha)								Yield attributing parameters				Economics				
	Cost of pruning (Rs./ha)	Cost of manure (Rs./ha)	Cost of fertilizers (Rs./ha)	Cost on field operations (Rs./ha)	Cost on chemicals (Rs./ha)	Cost on bioagents /botanicals (Rs./ha)	Cost on micro-nutrients (Rs./ha)	Total cost (Rs./ha)	Av. No. of marketable fruits	Av. Size of fruit (g)	Average yield (kg/plant)	Average yield (t/ha)	Rate of fruits (t)	Total returns (Rs./ha)	Total cost (Rs./ha)	Net returns (Rs./ha)	B:C ratio
Integrated strategy	2250.00	7500.00	4545.00	30000.00	3843.75	2937.50	2500.00	53576.25	105.58	190.00	20.06	10.03	20000	200600.00	53576.25	147023.75	3.74:1
Farmers practice	2250.00	6000.00	7000.00	30000.00	5606.25	293.75	4500.00	55650.00	71.37	190.00	13.56	6.78	20000	135600.00	55650.00	79950.00	2.43:1

IDM demonstrated plot



Single plant with fruits in IDM

Plate 19: Demonstration of IDM

5. DISCUSSION

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*, once a disease of minor importance took heavy toll during 2002. The severe outbreak of the disease in all the pomegranate growing regions of Maharashtra, Karnataka and Andhra Pradesh resulted in heavy yield losses both in terms of quality and quantity. Pomegranate, the boon commercial fruit crop to the farmer, turned as a big bane after the severe incidence of the disease was noticed. 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 on 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. High yield losses (60-80%) due to the disease was noticed by Chand and Kishun (1991) at IIHR experimental plots in Bangalore.

5.1 Survey and surveillance of bacterial blight of pomegranate

Survey on the incidence and severity of bacterial blight of pomegranate reveals the magnitude of the problem on hand and serves as a precursor for evolving the management strategies. Sudarshanrao (1975) stated that, survey and surveillance 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, fixed plot survey was undertaken for two years in major pomegranate growing areas of Karnataka and border villages of Andhra Pradesh 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 pooled results of two years survey, it was observed that, fruits were more vulnerable to the attack by bacterial blight than leaf as evidenced by more disease incidence and severity on fruits, irrespective of season, location and variety. Among the different districts under survey, maximum fruit infection of 38.29 per cent was recorded in Chitradurga district followed by Anantapur (36.64%) district of Andhra Pradesh, Koppal (32.40%) and Bellary (32.21%) districts. Correspondingly, average severity of the disease on fruits was observed as maximum in Bijapur district (28.95 PDI) and minimum severity of 17.86 PDI was recorded in Bellary district.

Similarly, average leaf intensity of the disease ranged between 16.17 to 26.33 PDI with highest leaf severity of 26.33 PDI was observed in Bijapur district. On the contrary, lowest disease index on leaf was recorded in Bellary district. 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.

Yenjerappa *et al.* (2004) recorded the highest severity of bacterial blight of pomegranate to an extent of 71.40 per cent in the villages of Bellary taluk and moderate incidence of 53.80 per cent in Bellary adjacent villages of Andhra Pradesh. On the contrary, in the present survey work, minimum fruit and leaf severity of 17.86 and 16.17 per cent, respectively was recorded in Bellary district, because most of the farmers in both the years had grown their crop during Hastbahar (September to March) during which weather, conditions such as no rainfall from third week of November till the harvest of the crop (in both the years) and low minimum temperature between 14.8 to 23.0^{0}C (2006) and 10.2 to 21.3 (2007), which were found unfavourable for the disease development and spread during growth period of the crop. However, the minimum disease severity on leaf and fruit was observed due to favourable environment for a shorter period (intermittent rainfall and minimum temperature of 19.1 – 22.8^{0}C during 2007) from September to October. Similar reasons for low disease incidence and severity (bacterial blight of pomegranate) in hastbahar were attributed by Yenjerappa *et al.* (2004). Contrary to this maximum disease severity was

recorded in Bijapur district. The reason being that the crop by most of the farmers was grown during mrigbahar followed by ambiabahar, which were the most vulnerable seasons for the attack of bacterial blight due to the existence of favourable environment such as continuous intermittent rainfall, optimum maximum temperature between 36.5 to 42.9^{0}C and minimum temperature between 20.8 to 24.2^{0}C (Yenjerappa *et al*., 2006).

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 varieties, Bhagwa was found more susceptible with more average disease severity on leaf (20.05 PDI), stem (2.08 lesions/branch) and fruits (22.71 PDI). The disease severity on fruit was comparatively less on Ganesh (15.09 PDI). Among the seasons, mrigbahar was found more vulnerable than ambiabahar and hastbahar.

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 also grew, increased their size (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 shed off finally. 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 hanged in the plant itself.

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 narrated 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 all the isolates collected from different areas yielded well separated, typical, yellow, mucoid bacterial colonies on nutrient agar medium after 72 hours of incubation at 30^{0}C. Culture of each isolate was purified by streaking suspected single colony on to the yeast dextrose calcium carbonate agar medium. All the 20 isolates produced pale yellow to dark yellow colonies with mucoid and convex appearance on YDCA medium. Pathogenicity of all the isolates 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 has 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.

The observations made pertaining to the isolation and pathogenicity in the present investigation were in conformity with the work of Manjula (2002), who obtained pure culture of the seven isolates of the pathogen from infected plant parts on nutrient agar medium by dilution plating technique and pathogenicity of all the isolates were proved by spray method of inoculation using 45 days old pomegranate plants of variety Jyothi.

5.4 Identification of the pathogen

Identification of the pathogen was determined with the studies on its morphological, biochemical, cultural and physiological features of the pathogen.

5.4.1 Morphological characters

Studies on morphological characteristics of the pathogen indicated that the bacterium was rod shaped with rounded ends occurred singly or rarely in pairs, gram negative, capsulated, non-spore forming with single polar flagellum. The cells measured 0.4 to 0.25 $\times$ 1.25 – 3.0 µm in size and were readily stained with crystal violet, gentian violet and carbol fuchsin. The results obtained in the present study on morphological characters were in agreement with the reports of earlier workers, Hingorani and Singh (1959), Kanwar (1976) and Manjula (2002).

5.4.2 Biochemical characters

With the results of present study, it is observed that, the pomegranate bacterium can hydrolyse the starch, liquefied the gelatin and is positive for H_2S production, catalase and oxidase enzyme activity. The organism utilized various carbon sources *viz.,* glucose, fructose, dextrose and produced mild acid from these carbon sources, but fail to utilize lactose, mannose and mannitol. Some of these biochemical characteristics identified in the present investigation were in accordance with the results obtained by Hingorani and Singh (1959) and their finding included, that the pomegranate pathogen utilizes xylose, glucose, mannose, galactose, sucrose, lactose and raffinose but not maltose, glycerine and salicin. It readily hydrolyses the starch and liquefies the gelatin. The yellow colour of the growth on gelatin gradually changes from usual bright yellow to dark brown on yeast glucose chalk agar and cooked potato. This discolouration is a specific character and in view of this character, the authors designated the pathogen as *Xanthomonas punicae* sp. nov.

Chand and Kishun (1991) reported that the pomegranate bacterium was positive to Xanthomonadin, tween 80 hydrolysis, gelatin liquefaction, milk proteolysis, H_2S production, catalase and the bacterium found to utilize glucose, fructose, mannose, sorbose, ribose *etc.* most of these biochemical characteristics were found similar in the present investigation except that, the pathogen failed to utilize mannose, mannitol and lactose as carbon sources.

As per the reports by Vauterin *et al.* (1995), *Xanthomonas axonopodis* can be distinguished from other *Xanthomonas* spp. with the presence of metabolic activity on different carbon substrates *viz.,* dextrin, cellobiose, maltose, gentibiose, D-trehalose *etc.* but lack of metabolic activity on other carbon substrates such as L-rhamnose, galacturonic acid, alanine, thymine *etc.* and he designated the pathogen causing leaf spot of pomegranate as *Xanthomonas axonopodis* pv. *punicae*. The results obtained in the present study also confirm the effective utilization of cellobiose in MD-5 medium by the pathogenic organism.

5.4.3 Cultural characteristics of the pathogen

Every living creature on this earth requires food for its growth and further life processes. Bacterium is not an exception to it, which secure the food from the substrate either plant or animal material, besides, they can also be cultured on artificial media provided with the supplement of essential nutrients. All the media may not equally support the good growth of all kinds of bacteria. So, specificity of the medium in question for the growth of *Xanthomonas axonopodis* pv. *punicae,* different media including differential and selective were tried in the present experiment.

Of the various media tested for their efficacy to support the growth of *X. axonopodis* pv. *punicae,* modified D-5 medium was found superior in promoting good growth as maximum bacterial colonies (150.75×10^5 cfu/ml) were recorded followed by yeast extract nutrient agar medium (110.75×10^5 cfu/ml). The next best supportive media includes GYCA, tween 80 and nutrient agar. Poor growth of the pathogen was recorded in BSCAA medium as evidenced by minimum number of bacterial colonies (10×10^5 cfu/ml).

Although, colony diameter ranged between 0.5 mm to 4.0 mm, medium to large sized colonies between 2 to 4.0 mm were observed on YNA, GYCA and MD-5 medium, colony diameter was very small (0.5 – 1.0 mm) on XTS agar medium.

Present findings pertaining to the growth of *X. axonopodis* pv. *punicae* on different media can be well comparable with the report by Hingorani and Singh (1959). They opined that, nutrient dextrose agar, yeast glucose chalk agar and potato cylinders are the best media for the cultivation of pomegranate bacterium. Similarly in the present work, the differential media amended with yeast (YNA, GYCA, YDC) and nutrient agar supported the good growth of the organism.

Colony characteristics of the pathogen differed with respect to media. It is observed that, circular to irregular, flattened, light yellow to brown coloured colonies, occurred singly but rarely in aggregate on MD-5 and YNA medium. Colonies with similar morphology but bright yellow colour appeared on GYCA and YDC medium. The bacterium having slightly raised, mucoid, light yellow to dark yellow coloured colonies were recorded on nutrient agar and starch agar medium. Colonies were minute, slightly raised, circular and creamy white on XTS agar medium.

During 1991, Chand and Kishun recovered circular, convex and yellow coloured colonies of the pomegranate bacterium with mucoid and glistening characteristics on nutrient agar medium. They also reported that, the pathogen produced clear starch digestion zone on SX medium. On the contrary to the above observation, pathogen failed to grow on SX medium as per the observations of present investigation. On the similar lines Sushma Joseph (1997) recorded the highest recovery of the colonies of *X. axnopodis* pv. *dieffenbachiae* on tween medium followed by cellobiose starch medium.

5.5 Cultural variability

The knowledge on extent of variability in a given pathogen has a wide practical utility, which can be made out from differences in virulence and nutrition (Venugopal, 1983). All the microorganisms possess traits that can be used to distinguish them and often artificial culture media can capitalize on these characters to facilitate selection (Schaad and Stall, 1988). Muras *et al.* (1983) studied the natural variability of morphological, cultural and biochemical properties of culture collection of phytopathogenic bacteria and stated that, among more than 1000 cultures of *Pseudomonas*, *Xanthomonas*, *Corynebacterium, Erwinia* and *Agrobacterium* spp. three per cent showed variability in colony morphology. *Xanthomonas* and *Pseudomonas* spp. were the most sensitive to change under storage.

The extent of variability with regard to the growth characters of 20 isolates of *Xanthomonas axonopodis* pv. *punicae* collected from different geographical locations was assessed. Five different media were chosen for the study.

The bacterium possess an ability to utilize wide spectrum of nutrients as a source of energy. Among the five selective/semi-selective media tested in the present study, modified D-5 medium was found to support the maximum growth of many isolates followed by Tween

80 and XTS agar. Least growth of isolates was recorded on BSCAA medium and 15 isolates failed to grow on SX agar media.

Two selective media were developed for the isolation and preliminary identification of *Xanthomonas campestris* pv. *dieffenbachiae* (Meculloch and Pirone) Dye. the principal carbon sources were cellobiose and starch (Norman and Alvarez, 1989). Similarly in the present investigation, excellent growth of many number of isolates on modified D-5 medium may be attributed to their ability to utilize D-cellobiose as a major carbon source. Ammonium chloride as nitrogen source. Potassium phosphate and magnesium sulphate are the other nutrients required by the pathogen in the medium. The present findings derive support from the results of Raut (1990).

Tween medium also supported maximum growth of Xa14, Xa7, Xa20, Xa1, Xa11 and Xa17 isolates, which derived nitrogen from peptone as a major component of the medium. Prabhu (1982) studied the different factors for the growth of *Xanthomonas beticola* and reported that dextrose as the best carbon source and peptone as nitrogen source. Similarly, the isolates Xa11, Xa10, Xa2, Xa20 and Xa15 grew well on XTS agar utilizing glucose and peptone as carbon and nitrogen supplements, respectively.

As per the report by earlier workers (Watanabe, 1963, Venugopal, 1983 and Raut, 1990), bacteria are very specific in the utilization of carbon and nitrogen sources. Carbon is one of the essential elements and its utilization depends on enzyme system of bacteria, whereas nitrogen in the organic or inorganic form is also essential for their growth.

Poor growth of the isolates on BSCAA medium and maximum failure of isolates to grow on SX agar showed their inability to utilize starch, glycine and ammonium chloride. Similar results were recorded by Sushma Joseph (1997), while studying the nutritional requirements of *Xanthomonas campestris* pv. *dieffenbachiae*. On the contrary the isolates of *Xanthomonas campestris* pv. *mangiferaeindicae* recorded the maximum growth, when ammonium chloride and ammonium sulphate were added as inorganic sources of nitrogen in the basal medium (Raut, 1990).

5.5.1 Variation on colony morphology

Schaad (1992) reported that, most pathovars of *Xanthomonas campestris* can be differentiated by growth and colony morphology on semi-selective agar media like SX, SM, BSCAA, MXP, XPS, XCS, MD-5 and Tween media.

The morphological traits also indicated enough variation among the isolates of *X. axonopodis* pv. *punicae* on five tested media. On modified D-5 medium, the isolates differed greatly in respect of colony colours. The isolates Xa3, Xa4, Xa5, Xa7, Xa14 and Xa16 exhibited creamy white colonies and others have light yellow (Xa1, Xa6, Xa8) or light yellow to brown coloured colonies. Variation in size and shape indicated that ten isolates have small to medium sized colonies (1-2 mm and 2.1-3 mm in diameter) with an exception that colonies of Xa2, Xa5 and Xa9 were exclusively small (<1.0 mm in diameter) and an isolate Xa15 possessed larger sized colonies (3.1 – 4.0 mm). Colony appearance was either flattened (Xa1, Xa4, Xa7) slightly raised (Xa2, Xa4) or convex (Xa6, Xa8).

Raut (1990) observed the variation in colony characteristics of different isolates of *X. campestris mangiferaeindicae* on different media. The colonies of Xcmn-7 and Xcmn-11 isolates were found smooth, mucoid, glistening and dark yellow colour on sucrose peptone agar, while Xcmn-1 produced milky white colonies on the same media.

The colony characters of twenty isolates were also differed on Tween 80 medium. The colonies were yellow, pale yellow and creamy white in ten numbers of isolates. The shape of the colony is circular in maximum number of isolates. However, irregular colonies were produced by Xa6 and Xa18 isolates. The colonies were slightly raised or convex, mucoid with glistening in four isolates and five isolates have flattened colonies.

The isolates Xa6, Xa10, Xa11, Xa12 and Xa15 exhibited very small, circular to irregular and creamy white colonies on XTS agar. However, the colonies were yellow, convex or slightly raised in seven isolates. Fifteen isolates fail to grow on SX agar. On BSCAA medium, colonies of Xa2, Xa5, Xa9 isolates differed from other isolates as small, circular and dull white in appearance. The results of the present investigation were in conformity with the studies conducted by Shobha (1998) on *Xanthomonas axonopodis* pv. *glycines*.

Morphological groupings among the isolates of pomegranate bacterium were made using cultural characters on modified D-5 medium. The isolates were categorized into five main groups. G-II was more predominant with highest frequency of isolates having medium sized, circular to irregular, flattened and creamy white colonies. On the contrary, isolates of group-II found to possess small to medium sized, slightly raised, light yellow to light brown coloured colonies.

On the similar lines, Jindal and Patel (1984) studied the variation among the 83 isolates of *Xanthomonas* on the basis of cultural characteristics. They distinguished the isolates into four category types *viz*., big raised mucoid opaque (BRO), small raised mucoid opaque (SRO), small slightly raised translucent (SRT), small flat dry translucent (SFT).

5.6 Molecular variability

The molecular technique, random amplified polymorphic DNA (RAPD) was used to detect the variations among the 20 isolates of *Xanthomonas axonopodis* pv. *punicae*. In the present study, the group of primers belonging to OPA, OPB and OPF series were used to determine the genetic differences among the isolates and to construct a dendrogram. Among the 20 primers used 11 primers exhibited amplification. The primers, OPA 20, OPB 03, OPF 07 and OPF 10 showed 100 per cent polymorphism that helped to identify the isolates with certain degree of confidence and served as a basis for identification of specific primers.

The information related to the banding pattern of all the primers that were used to detect the genetic distance among the isolates and to construct a dendrogram revealed the significant molecular variability. Based on the simple matching coefficient, genetic similarity matrix was constructed, which revealed the degree of relatedness among the isolates. The similarity coefficient ranged from 0.47 to 0.81. The maximum genetic similarity of 81 per cent was observed between Xa3 and Xa6 isolates followed by 80 per cent similarity between Xa9 and Xa19, 79 per cent similarity between Xa14 and Xa3 isolates, whereas least genetic similarity of 47 per cent was noticed between Xa12 and Xa1, Xa15 and Xa1 isolates.

Further, the dendrogram obtained out of RAPD data revealed that, the 20 isolates of pomegranate bacterium were differentiated into two major sub-clusters A and B. Cluster B was divided into single sub-cluster representing isolates Xa1 and Xa18 from Bellary and Bijapur districts, while major cluster A composed of isolates belonging to Bijapur (Jumnal), Bellary (Kurgod, Uppinayakanahally, Byasidageri, Shivanandanagar, Kappagal and Halakundi), Bagalkot (Kaladagi and Ankalagi), Koppal (Kustagi, Guttur, Yalaburti and Yalburga), Chitradurga (Hiriyur and Mollakalmuru) as well as adjoining parts of Andhra Pradesh (Hirehal and L.B. Nagar). From the results on variability, it was amply clear that, isolates of same geograohical area were not always closely related but distinct and differed with each other with considerable variability between them, which may be attributed to genetic environment and their interactions.

The RAPD technique has wide spread acceptance because it is relatively simple, well suitable assay when the nucleotide sequence is unknown. On the contrary, Chakrabarty *et al.* (2004) reported the suitability of RFLP technique to differentiate the strains of *Xanthomonas axonopodis* from different geographical regions of the world as the technique exhibited wide degree of polymorphism and clustered them in three different groups. In addition to interracial variability, a clear evidence of intraracial variability was detected within race 18 isolates of *Xanthomonas malvacearum*.

Siraree *et al.* (2004) generated the RAPD profiles using single decamer primers in polymerase chain reaction to study the diversity in *Xanthomonas campestris* pv. *campestris* population prevalent in the region. They standardized the amplification conditions so as to get polymorphism specific for individual isolate. Amplification products ranged from 6 to 12 with fragment size ranging from 100 bp to 5.0 kb.

5.7 Physiological characters

Under varied temperature levels tested for the growth of *X. axonopodis* pv. *punicae*, the temperature of 30^0C was found optimum for the maximum growth of the pathogen, although, it grew at a wide temperature levels ranged between 15 to 45^0C. No growth of the pathogen was recorded at highest and lowest extreme temperature levels of 50 and 5^0C, respectively.

The data related to the effect of pH for the growth of *X. axonopodis* pv. *punicae* revealed that, the growth (number of colonies) of the pathogen was found increased with increase in pH from 4.0 to 7.0 and beyond which, growth decreased as the pH of the medium further increased. The number of bacterial colonies recorded at pH 7.0 were significantly higher (145.33×10^5 cfu/ml) than the colonies at other levels of pH. Colonies counted were significantly least at lowest and highest pH levels of 4.0 (10×10^5 cfu/ml) and 10.5 (4×10^5 cfu/ml) and the pathogen failed to grow at pH levels of 3.0 and 11.0.

Similar work on temperature requirement was carried out by Hingorani and Mehta (1952). They found that the pomegranate bacterium grow well at a cordial temperature of 30^0C and can tolerate a minimum and maximum temperature of 5 and 40^0C, respectively. Gour *et al.* (2000) also got the similar results while working with *X. axonopodis* pv. *vignicola*, the causal agent of leaf blight of cowpea. They have recorded the maximum growth of the pathogen at a temperature level of 30^0C and pH of 7.0. Growth (number of colonies) declined considerably at pH values higher and lower than 7.0 being minimum at a pH of 5.0. Manjula (2002) recorded the highest number of colonies of *Xanthomonas axonopodis* pv. *punicae* at a temperature of 27^0C and pH of 7.2.

5.8 Disease epidemiology

5.8.1 Effect of dates of pruning and role of weather factors on bacterial blight development in pomegranate

Effect of seasonal variation in temperature and humidity on bacterial leaf blight development in pomegranate was determined by Hingorani and Singh (1959). They recorded the successful infection of the disease only from middle of March to the end of June and severity of disease was attributed to the prevalence of high temperature and low humidity. In the present investigation, the pomegranate crop grown during different seasons (pruned during different months) was observed for bacterial blight development in relation to weather factors. In late hastbahar season, when the crop was pruned in the month of November 2006, disease on fruit was noticed during 17^{th} week of 2007 and continued to progress further and reached its peak severity during 28^{th} week. Leaf infection was noticed bit late (21^{st} week), but progressed till the end of cropping season (32^{nd} week).

Similarly, when the crop was grown in ambiabahar cropping season (pruned in April 2007), initial infection was recorded during twenty fourth week of 2007, gradual disease progress was observed till 38^{th} week and disease came down gradually in subsequent weeks and reached the lowest severity in 44^{th} week. Progress of the disease, however bifurcated in the above seasons right from 17^{th} week of 2007 till 38^{th} week was attributed to the conducive weather factors such as rainfall in most of the weeks, required maximum ($29.4 – 41.2^0$C) and minimum ($19.5 – 27.3^0$C) temperature and relative humidity of 68.0 to 86.0 per cent, which favoured the disease development. The gradual reduction of the disease (in ambiabahar season) from 39^{th} week till the end of cropping season in 44^{th} week was mainly due to the prevalence of uncongenial minimum temperature ($12.1 – 21.2^0$C) and very less rainy days. However, wide variation in disease intensity observed in the similar weeks (21^{st} to 28^{th} week) of the above two seasons was mainly due to the difference in growth stage of the crop *i.e.*, in late hastbahar cropping season, when the crop was pruned in the month of November 2006, plants require two months time for giving rise to foliage, flowers .and fruits and further owing to uncongenial environment from 1^{st} to 16^{th} week, infection was delayed. Similarly in ambiabahar cropping season, when the plants were pruned in the month of April 2007, infection was delayed for the further period of 11 weeks due to unfavourable environment and early stage of the crop growth.

The above results can be well compared with the epidemiology of black arm of cotton studied by Lokhande and Newaskar (2000). They reported that, the disease occurred during August and September on two months old crop under the favourable weather conditions of 300 to 350 mm rainfall, mean temperature of 22.80 to 31.5^0C and relative humidity between 58 to 78 per cent. Infection rate was found declined from November onwards, owing to no rainfall, decrease in mean temperature ($19.1 – 30.3^0$C) and relative humidity (40% – 78%). According to Atulchandra *et al.* (1994), the disease caused by bacteria on pomegranate spreads very fast due to high temperature and low humidity from March to July.

Further in mrigbahar cropping season (pruned during July, 2007), disease symptoms were appeared at the end 40th week of 2007 continued to progress till the end of 48th week. Later on incidence and severity of the disease gradually reduced from 49th week and reached the lowest amount by 8th week of 2008.

Similarly in hastbahar cropping season (crop pruned during September 2007), disease was first noticed on leaf during 43rd week of 2007, while on fruit, initial symptoms of the disease were noticed during 45th week. Then, slowly progressed for a shorter period of subsequent two weeks, gradually decreased from 49th week and crop was found completely free of infection from 1st week of 2008 to till the harvest of the crop (9th week of 2008). Wide variation in disease intensity of the above two different seasons but in similar weeks from 45th to 52nd week was mainly due to the difference in growth stage of the crop as discussed in other two seasons *viz*., late hastbahar and ambiabahar.

Looking into the weather factors of both the seasons, there was a favourable environment for the disease to progress from 37th to 48th week of 2007. Intermittent rainfall of 4.5 to 91.8 mm, accompanied with congenial maximum (29.7 – 35.6^{0}C) and minimum (12.1 – 21.8^{0}C) temperature and relative humidity of 63.6 to 87.1 per cent prevailed during the period favoured the disease development. On the other side, declining of disease from 49th week onwards might be due to the non-receipt of rainfall and lower minimum temperature (10.2 – 21.3) as an uncongenial environment.

From the results obtained over the seasons, inference can be drawn that, the environment which prevailed from 29th to 44th week of 2007 was very conducive for the development and spread of bacterial blight in pomegranate. On the contrary, the weather factors which prevailed from 45th to 8th week of 2008 were highly uncongenial for the disease to progress. Hence, there was a disease reduction.

Rangaswamy (1975) and Singh (2000) reported that, temperature between 25 to 30^{0}C, high relative humidity and moderate rainfall as favourable weather factors for the development of black arm of cotton.

Report by Chand *et al.* (1991b) revealed that, pruning of grape vineyards from 1 to 15th September as the most vulnerable period for the attack of bacterial canker disease, which was against the findings of the present investigation that, September pruning is safer against bacterial blight of pomegranate the reason being, the difference in cropping pattern of each of these crops, although the weather relationship of both the diseases may be similar.

Srivastava and Bais (1985) recorded the maximum development of bacterial pustule of soybean in the month of August and September and was attributed to the favourable environment such as optimum temperature of 27^{0}C, relative humidity of 84.6 per cent and intermittent rainfall which corroborates the findings of present investigation.

Studies conducted by Yenjerappa *et al.* (2006) revealed that, the pomegranate crop, which was pruned during first and second fortnight of September was almost free from bacterial blight incidence from pruning to harvest except that very negligible disease intensity on foliage was recorded in the beginning of the cropping period. The reason given by the authors was uncongenial weather such as low minimum temperature (10.8 – 19.4^{0}C) and no rainfall received (November – March) during the growth and development stages of the crop. The findings are similar to the results of present investigation.

On the contrary, the crop pruned in the month of November was absolutely free from bacterial blight infection at early stages of its growth from December to March owing to uncongenial weather prevailed, but disease started progressing from April onwards with the receipt of unusual rains and prevalence of higher temperature during April and May (maximum temperature ranged between 36.5 – 42.9^{0}C and minimum temperature between 20.8 – 24.2^{0}C). The disease severity coincided with the fruit development and fruit maturity stage, where 90 per cent of developing fruits got infected resulting the huge loss in yield and quality.

5.8.1.1 Correlation with weather factors

Correlation coefficients of different weather factors with disease development revealed that, the maximum temperature had significant negative correlation with disease progress/decline in all the seasons except in ambiabahar, during which non-significant

positive correlation was established between maximum temperature and disease development both on leaf and fruit

Role of minimum temperature was significant and negatively correlated with all the disease parameters in late hastbahar and mrigbahar cropping seasons. Whereas, in ambiabahar season, the relationship was non-significant.

Effect of relative humidity and rainfall (late hastbahar and ambiabahar seasons) had both positive and negative relationship with disease development. Both of these factors have non-significant and positive correlation with disease incidence and severity on fruits whereas disease severity on leaf was negatively correlated.

In mrigbahar and hastbahar season, both relative humidity and rainfall had significant and negative relationship with all the disease components except that per cent incidence on fruit in hastbahar had significant positive relationship with relative humidity.

The results are in agreement with the work of Ravikumar (1997), who established the positive correlation between relative humidity and rainfall with incidence and severity of bacterial leaf spot of tomato.

Simple correlation coefficients established between bacterial leaf spot of tomato with weather variables revealed the significant and positive correlation with temperature, relative humidity and soil moisture (Shukla and Gupta, 2005). On the contrary, in the present investigation, almost significant negative correlation was observed between disease development and temperature, both positive and negative correlation was found with relative humidity and rainfall in different seasons.

Observed and estimated disease incidence and severity both on leaf and fruit during two major seasons mrigbahar and hastabahar followed the close relationship and significantly correlated with weather factors, indicating that the prediction models are fit and reliable. On the contrary, in ambiabahar season, observed and estimated values of all the disease components (incidence and severity) followed the wider range and non-significantly correlated with weather factors.

Step-wise regression models were developed taking disease components (incidence/severity) as dependent variables and weather parameters as independent variables. These models showed strong and significant positive relationship between the combined effect of weather parameters for disease development/decline with R^2 values ranged between 0.60 to 0.90 indicating 60 to 90 per cent forecastability of the disease (either incidence or severity on leaf or fruit) in major two seasons such as mrigbahar and hastbahar.

The results can be better compared with the findings of Shukla and Gupta (2005) on epidemiological aspects of bacterial leaf spot of tomato. They derived the multiple regression equation and emphasized the strong significant positive relationship between combined effect of weather variables (temperature, relative humidity, rainfall and soil moisture) and bacterial leaf spot severity in tomato.

5.8.2 Survival of the pathogen

The vital role of fallen leaves in the survival of phytopathogenic bacterium causing leaf spot of various crops was studied by Burkholder (1948).

With the results of present investigations, it is commonly noticed that, the pathogen *X. axonopodis* pv. *punicae* survives for little longer period, when the infected residues were buried in sterilized soil condition in comparison with unsterilized soil condition. The reason being the no competition by other organism when the soil condition is sterilized, but the similar condition does not exist practically in the field. Besides, population of the bacterium was found gradually decreased as the period of burying increased irrespective of sterilized or unsterilized soil condition and type of infected residues either leaf or fruit.

Under sterilized soil condition, the pathogen in the infected leaf residues survived for 20 weeks, when the residues buried just below the soil surface and at a depth of 5 cm. At a depth of 10 and 15 cm in soil, the pathogen survived for 18 and 16 weeks respectively. Similarly, on fruit residues, the survival ability of the pathogen was found little longer upto 22 weeks just below the surface of the soil and at a depth of 5 cm. At 10 and 15 cm depths of soil, the pathogen survived for 20 weeks. In the leaf and fruit residues buried under

unsterilized soil condition, the pathogen survived to the maximum if 18 and 20 weeks, respectively.

Hingorani and Singh (1959) working on survival ability of the pomegranate bacterium, determined that the pathogen survived on fallen leaves from December to mid March (16 weeks) and reproduced from March to end of June. The results obtained by them confirmed the present findings.

According to Rangaswamy (1962), the leaf spot causing bacterium on pomegranate infects through wounds and stomatal openings and could survive in soil for four months and cause fresh infections on new flush.

The results of present investigations were in better comparison with the results obtained by Shobha (1998) relating to the survival of *Xanthomonas axonopodis* pv. *glycines* causing bacterial pustule of soybean. The author could recover the bacterium through isolation beyond 20 weeks in the infected residues left on surface of the soil and upto 16 weeks in the residues buried at 10 cm depth.

Upasana Rani and Verma (2002) observed, still longer survival ability of the *X. axonopodis* pv. *punicae* upto 210 days in the infected fallen leaves kept under protected field condition. They also studied the pathogen lived in canker lesions upto 80 days such variations are attributed to geographical locations and environmental conditions.

5.8.3 Host range studies

Host range studies were conducted to know the ability of the test pathogen to infect the other plants. Among the several weed hosts and perennial plants inoculated artificially in the present investigation, the pathogen *Xanthomonas axonopodis* pv. *punicae* successfully infected neem, tridax and achyranthes plants in addition to its main host pomegranate. The disease symptoms on these plants appeared after 10 to 12 days of inoculation in the form of necrotic lesions, which later on developed into light brown to black coloured spots with blightning appearance.

The reports on host range by Hingorani and Singh (1959) and Manjula (2002) indicated that, the pathogen *X. axonopodis* pv. *punicae* was host specific and failed to infect the other plants.

The results are in confirmity with the findings of Ravikumar *et al.* (2005), who succeeded in proving the host range of the pomegranate bacterium.

5.9 Integrated disease management

5.9.1 Efficacy of bleaching powder and Bordeaux mixture in reducing the initial inoculum of the pathogen

Bleaching powder and Bordeaux mixture are the age old antibacterial chemicals used for the control of plant pathogenic bacteria. Efficacy of stable bleaching powder in reducing black rot of garden stocks (Wilson, 1942) and black rot of cabbage (Patel *et al*., 1950) was reported by earlier workers. Thind and Payak (1972) reported that many plant pathogenic bacteria had high degree of sensitivity to chlorine as stable bleaching powder.

The present investigation was aimed at assessing the efficacy of these two chemicals in reducing the initial inoculum of *X. axonopodis* pv. *punicae.* Results indicated that, Bordeaux mixture 1% spray was highly effective in eliminating the pathogen completely from the treated foliage as there was no recovery of the pathogen upon isolation from treated foliage. Foliar spray of copper oxychloride (0.2%) along with soil application of bleaching powder (100 g/plant) was found next effective treatment. Foliar application of copper oxychloride (0.2%) or soil application of bleaching powder (100 g/plant) as separate treatments were found moderately effective in reducing the pathogen population. Report by Rangaswamy (1962) revealed that, bacterial disease on pomegranate can be effectively managed with the sprays of 5:5:50 Bordeaux mixture or perenox (1%) or 1:50 lime sulphur. According to Thompson (1965), spread of bacterial stalk rot of corn could be effectively checked by chlorination of water through sprinkler irrigation. Stable bleaching powder was found quite effective in reducing the colonies of *Xanthomonas campestris* (Chand *et al*., 1981).

Similar results on efficacy of Bordeaux (5:5:50) used in combination with either streptocycline (500 ppm) or plantomycin (500 ppm) in reducing citrus canker (*Xanthomonas citri*) were obtained by Krishna and Nema (1983). Spraying of Bordeaux mixture alone also recorded the least canker incidence in comparison with bavistin (0.1%).

Findings of the present investigation, derived support from the report of Jadhav and Sharma (2009), who recommended the application of bleaching powder or copper dust (copper oxidase 4%) @ 20 kg/ha and spraying of Bordeaux mixture (1%) at monthly interval during of *Xanthamonas axonopodis* pv. *punicae* causing bacterial blight of pomegranate.

5.9.2 Effect of stem treatment of bactericides against the infection on stem

Stem infection of pomegranate by bacterial pathogen (*X. axonopodis* pv. *punicae*) is well established as a nodal blight (Chand and Kishun, 1990), through which infection spreads from one season to another. Eradication of infection on stem/branches is often difficult either by mechanical means or by foliar application of bactericides, because, stem is an integral part of the plant and can not be cut completely while pruning and foliar application of bactericides against stem infection is not practically feasible.

Hence, as an alternate option to prevent the spread of inoculum from stem, study was taken up to evaluate the efficacy of different bactericides by the way of pasting these bactericides on the infected surface of stem/branch.

Results recorded at 60 days after pasting revealed that, black out, bronip and bactrinashak each one @ 0.05% concentration in combination with COC were equally effective in reducing the spread of inoculum as significantly lowest disease incidence was recorded in the treated plots of these bactericides. Correspondingly, disease severity was significantly reduced in bronip + COC (5.79 PDI) and streptocycline + COC (7.76 PDI) treated plots.

Although, the results in the similar trend were recorded 90 days after pasting these bactericides, but the incidence and severity of the disease were increased in all the treatments in comparison with the previous observations. However, among the different treatments, black out and bronip each in combination with copper oxychloride significantly recorded least incidence of 24.90 and 30.80 per cent, respectively. In respect of disease severity, bronip (9.95 PDI) and streptocycline (12.68 PDI) each in combination with COC were found highly effective in reducing the severity. Stem pasting with copper oxychloride (0.2%) alone was found least effective. Consequently higher on par yield of 9.81 and 8.43 tonnes per ha were recorded in bronip and black out treated plots, respectively.

Jadeja *et al.* (2000) recorded the highest canker control in citrus with the spray application of streptomycin sulphate (100 ppm) in combination with copper oxychloride (0.2%), while, stem pasting with Bordeaux paste was found ineffective against citrus canker. Spraying of Bordeaux mixture (5:5:50) alone or in combination with streptocycline or plantomycin was observed very effective against canker incidence in citrus (Krishna *et al*., 1983). Looking into the present findings and reports by earlier workers, stem treatment with bactericides is not as much effective as foliar applications in preventing or controlling the bacterial infections of fruit crops.

5.9.3 Evaluation of bactericides/antibiotics

5.9.3.1 *In vitro* studies

In vitro evaluation of pesticides or biopesticides 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 bactericides and antibacterial chemicals evaluated in the present investigation, 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.

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. These results are similar to the findings of present investigation as the technical grade and composition of bactrinol, bacteriomycin, bronip, bactrinashak are same *i.e.*, 2 bromo 2 nitropropane 1-3 diol.

5.9.3.2 Field evaluation of bactericides and antibacterial chemicals against bacterial blight of pomegranate

The study was conducted for two different seasons Mrigbahar and Ambiabahar to assess the efficacy of commercially available bactericides/antibacterial chemicals for the management of bacterial blight of pomegranate.

Results of mrigbahar season trial indicated that, bronip (0.05%) + COC (0.2%) was significantly effective than all other bactricides in minimizing the disease incidence followed by bactrinashak, bactinash-200 and K cycline each at similar concentration and combination with COC. These bactericides, exhibited on par efficacy each other, however, their efficacy was significantly superior to streptocycline (0.05%) and plantomycin (0.1%). The antibacterial chemicals were found totally ineffective with >50 per cent incidence.

In respect of reducing the disease severity, all the bactericides except plantomycin were found equally effective with the record of lowest disease severity ranged between 3.71 to 4.69 PDI.

Kishun and Sohi (1984) reported that bacterial canker of mango could be effectively controlled by four sprays of bavistin (0.1%) or bavistin (0.1%) + agrimycin-100 (100 ppm). Similarly, 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.

In ambiabahar cropping season, the incidence and severity of the disease is generally less (before the treatment imposition) than mrigbahar season attributed to the weather factors, which may not be much favourable during the season as discussed in earlier chapters. However, in bactericides treated plots, the results recorded are almost similar to those obtained in mrigbahar season that bronip, bactnash-200 in combination with COC were significantly and highly effective in reducing both incidence and severity of the disease. In a similar way, bactrinashak + COC and streptocycline + COC were the next best effective bactericides. The efficacy exhibited by these bactericides was non-significant each other.

Performance of antibacterial chemicals in controlling the disease was very poor as these chemicals recorded, disease incidence of more than 50 per cent and severity ranged between 27.76 (bleaching powder) to 31.74 PDI (Bordeaux mixture).

Data on yield parameter revealed that, highest fruit yield of 9.72 tonnes per ha (mrigbahar) and 10.68 tonnes per ha (ambiabahar) was recorded in bornip + COC treated plot, followed by bactrinashak + COC (with the yield level of 8.90 t/ha and 9.65 t/ha) in Mrigbahar and ambiabahar cropping seasons, respectively) as influenced by effective disease control.

Suriachandraselvan *et al.* (1993) suggested the application of Paushamycin (0.05%) in combination with copper oxychloride (0.2%) for the control of bacterial disease on pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. Atulchand *et al.* (1994) stated that same bacterium could be effectively controlled with the sprays of Bordeaux

mixture (5:5:50) or any other copper fungicides at an interval of 15 days. Contradictory to this, performance of Bordeaux mixture was very poor in the present investigation against the disease probably due to the aggressive nature of the pathogen accompanied with favourable environmental conditions such as continuous/intermittent rainfall (in some weeks), maximum and minimum temperatures ranged between 27.7 to 35.8 and 12.1 to 24.9, relative humidity of 54.8 to 86.0 per cent which prevailed throughout mrigbahar season (2007), lead to the outbreak severity of the disease, which could not be mitigated easily with the sprays of Bordeaux mixture. The present findings on efficacy of streptocycline or other bactericides *viz.,* bronip, 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. The technical grade of bactrinol-100 is same as that of bronip/bactrinashak/bactinash-200 *i.e.,* 2 bromo-2nitro propane, 1, 3, diol.

Results pooled over the seasons revealed the significantly superior efficacy of bronip (0.05%) + COC (0.2%) than all the treatments in reducing the disease incidence. The next best effective treatments were bactinash-200 + COC, bactrinashak + COC, K cycline + COC and streptocycline + COC which recorded disease incidence ranged between 23.26 to 30.89 per cent.

Overall efficacy of all the three antibacterial chemicals was significantly low in reducing the disease incidence as evidenced with the record of >50 per cent incidence.

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 very low in all the bactericides treated plots except plantomycin (0.5%) + COC (0.2%) treatment indicating greater efficacy of all the bactericides.

Average yield pooled over the seasons revealed the highest significant yield of 10.20 tonnes per ha obtained with bronip + COC treated plot followed by bactrinashak + COC (9.28 t/ha) as an evidence of effective disease control in these two treatments. The next significant highest yield of 8.94 tonnes per ha was recorded in the treatments of bactrinashak 200 + COC. K cycline and streptocycline each in combination with COC recorded the moderate yield of 7.83 and 7.54 tonnes per ha, respectively. The yield recorded in plantomycin (0.5%) + COC (0.2%) treated plot was significantly lower than all other bactericides and non-significant with the yield levels of antibacterial chemicals (4.97 – 5.43 t/ha). Significantly lowest yield of 3.30 tonnes per ha was recorded in untreated check plot.

Least bacterial leaf spot incidence on grape vine was recorded in the plots treated with streptocycline or streptomycin sulphate each at 0.05% concentration (Ravikumar *et al*., 2002). Pruning of infected parts along with spray of copper oxychloride followed by four sprays of streptocycline (100 ppm) + copper oxychloride (0.3%) was suggested by Gopal *et al.* (2004) for the effective control of bacterial canker of acid lime. 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, the efficacy of streptocycline in combination with copper oxychloride was very much emphasized against bacterial blight control, but however on comparison, the efficacy of streptoclycine is non-significantly lower than other bactericides *viz.,* bronip/bactinash-200/bactrinashak in managing the disease effectively.

The results are similar with the reports of Ravikumar and Yenjerappa (2005), who achieved the effective control of bacterial blight of pomegranate with five sprays of bactrinashak (0.05%) in combination with copper oxychloride (0.2%) along with highest yield and maximum benefit:cost ratio. However, the efficacy of bactinashak stands next to bronip or bactinash-200 in controlling the bacterial blight of pomegranate in the present study.

5.9.4 Evaluation of bioagents

5.9.4.1 *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* (both exhibiting on par efficacy with each other) 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.

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.* (1991a).

Laha *et al.* (1992) stated that 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 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. On contrary to the report by earlier workers and findings of present investigation, Manjula (2002) examined the ineffective mechanism of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Xanthomonas axonopodis* pv. *punicae*.

5.9.4.2 Field evaluation of biocontrol agents

The trial was taken up during hastbahar cropping season of 2007-08, when the disease pressure was normally low due to uncongenial weather conditions.

Results obtained after third and fifth application of biocontrol agents revealed that, combined sprays of *Pseudomonas fluorescens* (0.5%) and *Bacillus subtilis* (0.5%) were significantly effective with the record of lowest incidence and severity of the disease followed by the individual sprays of same biocontrol agents. The other biocontrol agents *viz., Trichoderma viride, Trichoderma harzianum* and *Pseudomonas putida* were found less effective with more disease incidence and severity. Highest disease incidence (26.15%) and severity (8.08 PDI) was observed in untreated check plot after fifth application of biocontrol agents.

Use of biocontrol agents in the management of plant diseases is an age old practice and effectiveness of bacterial biocontrol agents against various crop diseases was reported by earlier workers. Kalita *et al.* (1996) reported the reduction of citrus canker by the sprays application of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus polymixa*. They recorded the least canker incidence with the treatment by *Bacillus subtilis*.

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

Consequent upon the effective disease control in *Pseudomonas fluorescens* + *Bacillus subtilis* treated plot, highest and significant yield (10.82 tonnes/ha) was recorded in the former treatment followed by *Bacillus subtilis* and *Pseudomonas fluorescens*. The other biocontrol agents recorded the significant lower yields between 5.25 to 6.25 tonnes per ha.

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. *vignaeradiatae*.

5.9.5 Evaluation of botanicals

5.9.5.1 *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, garlic extract followed by parthenium leaf extract, onion bulb extract and lantana leaf extract exhibiting 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.,* turmeric rhizome extract, zinger rhizome extract, clerodendron leaf extract and neem seed kernel extract were fail to inhibit the growth of the pathogen even at higher concentration of 10 per cent. Overall effect of plant extracts revealed 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.

Aqueous extracts of *Allium sativum* (clove) and *Artabotrys uncinatus* (leaves) evaluated against *Xanthomonas campestris* pv. *oryzae* revealed the superior efficacy of both the extracts by producing largest inhibition zone against the growth of the pathogen. The bactericidal properties of onion were attributed to the presence of alkaloids (Grainge *et al.,* 1985).

Srinivasachary (1995) reported ocimum plant extract as most effective botanical against the growth of *Xanthomonas campestris* pv. *mori* isolated from mulberry. On the contrary, poor efficacy of the similar plant extract was recorded in the present study against *Xanthomonas axonopodis* pv. *punicae*. 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).

5.9.5.2 Field efficacy of botanicals

Among the nine different plant extracts, each one screened at 10 per cent concentration, garlic and onion bulb extracts (after third and fifth spray) were found significantly effective in reducing both incidence and severity of the disease.

Among the rest of the plant extracts, although there is a incremental intensity of the disease from previous observations. Lantana, parthenium leaf extracts and zinger rhizome extract significantly recorded less disease incidence and severity.

Among all the botanicals, ocimum leaf extract was least effective in controlling the incidence and turmeric rhizome extract was least effective against severity and the efficacy exhibited by these two extracts was on par with untreated check plot.

With regard to the yield levels, significantly superior yield was obtained in garlic bulb extract followed by onion bulb extract, lantana leaf extract and parthenium leaf extract. The lowest significant yield was recorded in clerodendron leaf extract, which is on par with the yield of untreated control (4.84 t/ha).

Kirankumar (2000) reported the effectiveness of *Ocimum sanctum* against bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv. *vignicola*, undiluted extract was found quite effective against the disease and effectiveness decreased with dilution. On the reverse side, poor efficacy of *Ocimum sanctum* in controlling the bacterial blight of pomegranate was detected in the present investigation. Madhiazhagan *et al.* (2002) studied the field efficacy of five botanical extract in controlling the bacterial blight of rice and opined that, among the different extracts screened, *Adathoda vasica* significantly minimized the disease with higher grain yield levels. *Curcuma longa* and *Allium cepa* were the next best effective extracts. According to Rajeswari (1991), the antibacterial activity of *Adathoda vasica* and *Prosopis julifera* against bacterial pathogen is mainly because of high level of lycoprotein and tannin. The active principle behind the antibacterial properties of *Curcuma longa* and *Azadirachta indica* was reported to be the protein part of the plant extracts (Selvam, 1995).

Hence, the superior efficacy of garlic and onion extracts in reducing the bacterial blight disease of pomegranate as investigated in the present study was attributed to the presence of sulphur and alkaloids, which possess an antibacterial property as detected by the earlier workers.

5.9.6 Role of micronutrients on the incidence and severity of bacterial blight in pomegranate

Effect of application of micronutrients such as zinc, boron, magnesium, calcium, iron and multinutrients on the incidence and severity of bacterial blight of pomegranate was assessed in the field study.

Results obtained that, application of micronutrients in the form of either straight or multinutrients did not play any significant role towards the reduction of disease as the amount of disease was found increased with increase in number of sprays of micronutrients. However, looking into the effect of individual treatments, application of multinutrients significantly recorded the minimum incidence and severity of the disease (after second and fourth spray) followed by the application of calcium with on par incidence and severity of the disease.

The other micronutrients *viz.,* zinc, boron, magnesium and iron were found significantly ineffective with the record of more incidence than untreated check plot (57.49%). However, magnesium and iron treated plots recorded significantly least disease severity.

Impact of micronutrient on the fruit yield of pomegranate indicated that, significantly higher yield of 8.00 tonnes per ha was obtained in multinutrient treated plot followed by calcium. Significantly lower yield levels were observed in other treatments, which were found on par with the yield of untreated check plot (3.89 t/ha).

Strong correlation between uptake of boron in leaf tissues and residues of boron in soil with black rot incidence of cauliflower was reported by Kumar and Kotur (1989) and they opined that, deficiency and excess of boron in leaf tissues and soil induces the susceptibility of cauliflower to black rot caused by *Xanthomonas campestris* pv. *campestris* and no black rot incidence was recorded at the optimum level of boron between 0.4 to 1.6 mg per kg. Similarly in the present study, exogenous supply of boron either through soil or spray was found to favour the bacterial blight of pomegranate significantly.

Ansari and Shridhar (2001), studied the effect of iron on the virulence of *Xanthomonas oryzae* pv. *oryzae* and noticed that, increase in the concentration of iron on which, the pathogen was grown resulted the increased lesion length in both susceptible (IR-8) and resistant (DV-85) rice cultivars, when pathogen was inoculated artificially onto the rice seedlings. Pathogen grown in iron starved medium produced minimum lesion length. Similarly, in the present investigation, iron nutrition to the plants had no significant role in disease reduction.

5.9.7 Evaluation of IDM module

Control of plant diseases is most successful and economical when all the available methods of disease control are integrated suitably and implemented successfully. IDM module is essentially required to save the crop from unpredictable, sudden outbreak of diseases (Panday *et al*., 2005). Jadeja *et al.* (2000) achieved the successful control of citrus canker (*Xanthomonas axonopodis* pv. *citri*) with the adoption of integrated measures involving scraping of diseased portion on the main trunk, pasting with Bordeaux paste followed by the foliar application of streptomycin sulphate (100 ppm) + copper oxychloride (0.2%) three times a year. Similarly in the present investigation, IDM module evaluated against bacterial blight in pomegranate was found successful and fruitful with the record of least disease incidence (15.62%) and severity (8.47 PDI) as against the farmer method of disease control. Das and Shyam Singh (2003) recommended the integration of chemicals and cultural practices for the management of bacterial canker of acid lime.

The disease management performance of IDM module was reflected interms of yield, accompanied with net returns and B:C ratio as maximum average yield of 10.03 tonnes per ha accounting for higher net returns (Rs. 1,91,318.75) and benefit:cost ratio (21.6:1) was

realized with the adoption of IDM. On the contrary, lower yield levels (6.78 t/ha) lead to the less net profit of Rs. 1,25,200 was obtained in farmers method of disease control.

Panday *et al.* (2005) recorded the highest yield along with more net benefit with the adoption of IDM package against the four major diseases of tomato *viz.*, damping off, bacterial blight, alternaria blight and leaf curl and opined that, need based plant protection measures applied in IDM programme were cost effective and achieved economic yield with less environmental pollution than sole chemical methods. The outcome of present investigation is also attributed to the similar reasons. Hewson *et al.* (1998) stated that, level of disease control and crop yield in IDM programme are often better than conventional method. Thus, feasible integration and timely application of all the methods of disease control ensures successful plant protection.

Future line of work

1. Bioagents and botanicals may be tested under large scale trial.
2. Sources of resistance against bacterial blight need investigation.
3. Variability may be tested by other molecular methods
4. Disease prediction needs to be taken up.
5. Micronutrient application may be incorporated with other integrated measures for better efficacy.
6. IDM strategy may be evaluated in mrigbahar.
7. IDM needs to be taken on community basis.

6. SUMMARY AND CONCLUSIONS

Pomegranate (*Punica granatum* L.) belongs to the family Punicaeae is a vital cash crop of an Indian farmer. Among the different states cultivating pomegranate, Maharashtra is the largest producer followed by Karnataka, Andhra Pradesh, Gujrath and Rajasthan.

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 for two years revealed the highest disease incidence on fruits in the villages of Chitradurga district followed by Anantapur and Koppal districts. Correspondingly, highest disease intensity on both leaf and fruit was recorded in Bijapur district. Lowest disease incidence and severity was observed in the villages of Bellary district. Among the varieties, Bhagwa was found more susceptible and disease was severe in mrigbahar cropping season.

The disease manifested itself in the form of small water soaked brown to black coloured lesions on the upper surface of the leaves surrounded by a diffused water soaked margin on the lower leaf surface. As the disease progresses, the spots also grew, increased their size, coalesced and extended upto midrib by occupying major portion of the leaf lamina. Severely infected leaves turned yellow, became chlorotic and shed off finally. Stem infection was noticed in the form of long narrow, elongated brown to black coloured lesions on the main stem and branches. The lesions later on became rough and cankerours, leading to stem girdling and breaking at the point of infection. On flower buds, symptoms appeared as brown to black coloured spots, which later on coalesced and lead to the dropping of flower buds in advanced stages of infection. On developing green fruits small pin head sized black coloured lesions were seen with diffused water soaked margin surrounding the spot. Later on these lesions were turned to black coloured, medium to big sized spots. Severely infected fruits, split opened with L/Y/star shaped cracks. The infected fruits were dried up and hanged in the plant itself.

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. Pathogenicity of all the isolates was proved.

The bacterium was rod shaped with rounded ends occurred singly or in pairs, gram negative, capsulated, non-spore forming with single polar flagellum. The biochemical characteristics revealed that, the bacterium was positive to starch hydrolysis, gelatin liquefaction, H_2S production, catalase and oxidase. The organism utilized glucose, fructose, dextrose and failed to utilize lactose, mannose and mannitol.

Among the various media tested for their efficacy to support the growth of *X. axonopodis* pv. *punicae*, the modified D-5 medium was found superior in promoting the good growth followed by yeast extract nutrient agar medium. The next best supportive media were GYCA and Tween 80. Poor growth of the pathogen was recorded on BSCAA medium and no growth was observed on SX agar. Colony diameter ranged between 0.5 to 4.0 mm with large sized colonies were observed on MD-5 and YNA media. Colony characters of the pathogen were also differed in respect of media as circular to irregular, flattened, light yellow to brown coloured colonies on MD-5 and YNA media.

Cultural variability among the 20 isolates of the pathogen revealed the significant difference among the media in supporting the growth of different isolates of the pathogen. Maximum growth of fifteen isolates (colonies of $>70 \times 10^5$ cfu/ml) were recorded on modified D-5 medium followed by Tween 80 and XTS agar medium. Among the isolates, Xa7, Xa17,

Xa16 and Xa19 recorded significantly superior growth on modified D-5 medium. Tween 80 medium was found to support the maximum growth of Xa14, Xa7, Xa20 and Xa1 isolates and XTS agar supported the good growth of Xa11, Xa10, Xa2, Xa20, Xa17 isolates. The isolates did not grow well on BSCAA medium. However, the isolates Xa12, Xa13, Xa16 and Xa10 exhibited their moderate growth on BSCAA medium and fifteen isolates failed to grow on SX agar.

Isolates also differed in respect of colony characters. The isolates exhibited varied colouration. Creamy white or light yellow or light brown or yellow coloured colonies on different media. Size and shape of the colonies also differed as small, medium and large having circular to irregular in shape. The colonies of different isolates were either flattened, slightly raised or convex with or without glistening character. Morphological groupings among the isolates of the pathogen was made based on the similarity in colony characters on modified D-5 medium.

RAPD technique employed to detect the variations among the 20 isolates of the pathogen revealed the 100 per cent polymorphism for OPA20, OPB03, OPF07 and OPF10 primers. Out of 60 amplicon levels resulted from 11 primers, 51 amplicons were found polymorphic indicating that, there was a significant molecular variability among the isolates. The similarity coefficient ranged between 0.47 to 0.81, which indicated the degree of relatedness among the isolates. Further, the dendrogram showed two major clusters A and B. Cluster B was divided into single sub-cluster representing isolates Xa1 and Xa18 from Bellary and Bijapur districts, while the major cluster A composed of all other isolates belonging to different isolates of Bagalkot, Bellary, Bijapur, Chitradurga Koppal of Karnataka and Anantapur district of Andhra Pradesh.

Among the varied temperature and pH levels tested for the growth of *X. axonopodis* pv. *punicae* temperature of 30^0C and pH of 7.0 to 7.5 were found optimum for the good growth of the pathogen. The over and below of these levels decreased the growth.

Among the different seasons assessed for bacterial blight incidence and severity, mrigbahar (July-2007 pruning) and ambiabahar (April-2007 pruning) cropping seasons were found most vulnerable for the attack of bacterial blight, as the more disease incidence and severity coincided with the fruit development and maturity stage. Late hastabahar (November-2006 pruning) was found relatively safe because maximum harvest of the crop was over by the end of 20^{th} week, where disease incidence and severity was comparatively less. Growing crop during hastabahar (September-2007 pruning) was safe as the disease severity was les during initial stage of the crop and later on, crop was found completely free from disease till harvest due to unfavourable weather for longer period.

Continuous/intermittent rainfall for a longer period, congenial maximum (29.4 – 35.6^0C) and minimum temperature (19.5 – 27.3^0C) and relative humidity (63 to 87%) were found favourable for the development and spread of the disease.

Observed and estimated disease incidence and severity during mrigbahar and hastbahar followed the close path indicating that prediction models are fit and reliable during the seasons.

Correlation between disease and weather factors revealed that, maximum temperature had significant negative correlation with the disease development in all the seasons except in ambiabahar, where maximum temperature had non-significant positive correlation. Role of minimum temperature was significant and negative with the disease development in late hastbahar and mrigbahar season. Relative humidity and rainfall had positive and negative relationship with the disease in different seasons. Step-wise regression models showed strong and positive relationship between combined effect of weather factors for disease development or decline.

Studies pertaining to survival of the pathogen indicated that, the pathogen present in the infected leaf and fruit residues survived upto 20 and 22 weeks respectively, when these residues were buried in sterilized soil condition at different depths. The survival period was found little less upto 18 and 20 weeks in the infected leaf and fruit residues, respectively under unsterilized soil condition.

Among the several weeds inoculated to test the host range of the pathogen, the bacterium *X. axonopodis* pv. *punicae* successfully infected *Azadirachta indica*, *Tridax procumbens* and *Achyranthes aspera*. Reisolation and cross inoculation studies confirmed the host range.

Effect of bleaching powder and Bordeaux mixture on the reduction of initial inoculum of the pathogen showed that Bordeaux mixture (1%) spray was highly effective in eliminating the pathogen completely from the treated foliage. Foliar application of copper oxychloride (0.2%) along with soil application of bleaching powder (100 g/plant) was the next effective treatment.

Among the different bactericides evaluated to prevent the spread of disease from stem infection revealed that, blackout (0.05%) and bronip (0.05%) each in combination with COC (0.2%) recorded the least disease incidence. Similarly, bronip (0.05%) and streptocycline (0.05%) in combination with COC were found significantly effective in reducing the disease severity.

In vitro evaluation of bactericides and antibacterial chemicals indicated that, bronip (0.1%), bactinash-200 (0.1%) and plantomycin (1%) were highly effective in recording the maximum inhibition zone. Whereas in field evaluation over the seasons, bronip (0.05%) + COC (0.2%) was found highly effective in reducing the disease incidence followed by bactinash-200 + COC, bactrinashak + COC, Kcycline + COC and streptocycline + COC at the similar concentration. In respect of reducing disease severity, all these bactericides were found equally and significantly effective. The efficacy of all three antibacterial chemicals was significantly very low in reducing the disease incidence.

Average yield pooled over the seasons revealed significantly highest yield in bronip + COC treated plot followed by bactrinashak + COC for effective disease management.

In biological control, *Bacillus subtilis* and *Pseudomonas fluorescens* were significantly superior to other antagonists in inhibiting the growth of the pathogen. In field trial, similar biocontrol agents each at 0.5 per cent concentration were effective in reducing both incidence and severity of the disease and recorded significantly higher yield levels.

In vitro evaluation of different plant extracts revealed that, garlic extract (10%) produced the maximum inhibition zone followed by parthenium, lantana leaf extract and onion bulb extract each at 10 per cent concentration. Under field evaluation, garlic and onion bulb extract each at 10 per cent concentration significantly recorded the lowest disease incidence and severity of the disease. In respect of yield, garlic extract (10%) recorded significantly highest yield than all other plant extracts in the trial.

The role of micronutrients indicated that, none of the micronutrients applied in the form of either straight or multinutrients, played a significant role towards the disease reduction. However, comparison between different treatments revealed that, application of multinutrients at 1 per cent concentration, significantly recorded lower incidence and severity of the disease followed by iron and calcium (each at 1% concentration).

The IDM strategy evaluated for bacterial blight management in pomegranate was found successful with the record of lowest incidence and severity of the disease as against the farmers method of disease control. Adoption of IDM strategy recorded the higher yield (10.03 t/ha) in comparison with farmers practice (6.78 t/ha) accounting for high net returns and B:C ratio.

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APPENDIX

Appendix I: technical grade of bactericides and other chemicals

Sl. No.	Chemical name	Trade Name	Formulation	Company
1.	2 Bormo 2 nitro propane 1, 3, diol 95% WW	Bactinash 200	Powder	Multiplex Agricare Pvt. Ltd., Tumkur
2.	2 Bormo 2 nitro propane 1, 3, diol	Black out	Liquid	Multiplex Agricare Pvt. Ltd., Tumkur
3.	2 Bormo 2 nitro propane 1, 3, diol	Bactrinashak	Powder	Indofil Chemicals Company Ltd., Mumbai
4.	2 Bormo 2 nitro propane 1, 3, diol	Bronip	Powder	Gold Farms Plant Tech. Pvt. Ltd., Bangalore
5.	Streptomycin sulphate IP 90% WW + tetracycline hydrochloride 10% WW	Streptocycline	Powder	Hindustan Antibiotics Ltd., Pimpri, Pune
6.	Streptomycin sulphate IP 90% WW + tetracycline hydrochloride 10% WW	K cycline	Powder	Karnataka Antibiotics and Pharmaceuticals Ltd., Bangalore
7.	Streptomycin sulphate 9% WW + Tetracycline hydrochloride 1% WW	Plantomycin	Powder	Aries Agro Ltd., Mumbai
8.	Copper oxychloride 50% WP	Blitox	Powder	Rallis TATA Enterprises, Mumbai
9.	Copper sulphate + Lime	Bordeaux mixture	Liquid	-
10.	Chlorine 30%	Bleaching powder	Powder	Aqua Phoenix Solutions, Thane, Mumbai

Appendix II: Technical formulations of biocontrol agents

Sl. No.	Common name	Trade Name	cfu/g	Manufacturer
1.	*Bacillus subtilis*	*Bacillus subtilis*	2×10^8	UAS, Dharwad
2.	*Pseudomonas fluorescens*	Biocure B	1×10^8	T-Stanes Co. Ltd., Coimbatore
3.	*Pseudomonas putida*	*Pseudomonas putida*	2×10^8	Sujay Biotech Ltd., Bangalore
4.	*Trichoderma harzianum*	*Trichoderma harzianum*	2×10^6	UAS, Dharwad
5.	*Trichoderma viride*	Biocure F	2×10^6	T-Stanes Co. Ltd., Coimbatore

Appendix III: Rates of chemicals, bioagents, fertilizers and other inputs used in the study

Sl. No.	Particulars	Quantity	Rate (Rs.)
A.	Bactericides and other chemicals		
1.	Bactinash 200	20 g	50.00/-
2.	Bactrinashak	20 g	45.00/-
3.	Bronip	20 g	54.00/-
4.	Plantomycin	100 g	140.00/-
5.	Steprocycline	6 g	33.75/-
6.	K cycline	6 g	34.00/-
7.	Bleaching powder	1 kg	30.00/-
8.	Copper oxychloride	500 g	200.00/-
B.	Bioagents		
1.	Biocure B	1 kg	210.00/-
2.	Biocure F	1 kg	210.00/-
C.	Manures and Fertilizers		
1.	Farmyard manure (FYM)	1 tonne	300.00/-
2.	Vermicompost (VC)	1 tonne	2000.00/-
3.	Urea	50 kg	260.00/-
4.	Diammonium phosphate (DAP)	50 kg	500.00/-
5.	Muriate of potash	50 kg	260.00/-
6.	Complex (17:17:17)	50 kg	450.00/-

EPIDEMIOLOGY AND 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 and resultant losses due to bacterial blight in pomegranate, investigations were undertaken on disease, pathogen, environment and management aspects. Survey revealed the highest disease incidence in Chitradurga, Anantapur and Koppal districts and lowest incidence and severity was recorded in Bellary district.

The bacterium was rod shaped, gram negative and capsulated. It was positive to starch hydrolysis, gelatin, liquefaction and H_2S production. Modified D-5 medium was found superior in supporting the growth of the pathogen. Cultural variability among the 20 different isolates revealed the variability in growth and colony characters. The isolates exhibited 100 per cent polymorphism for OPA20, OPB03, OPF07 and OPF10 primers showing significant molecular variability. Among the different seasons, mrigbahar was found most vulnerable and hastbahar was found relatively safe in avoiding the disease. Rainfall for a longer period, maximum temperature between 29.4-35.6^{0}C and minimum temperature between 19.5 to 27.3^{0}C, RH of 63-87 per cent were found favourable for the disease development and spread.

Pathogen survived upto 20 to 22 and 18 to 20 weeks in the infected residues buried in sterilized and unsterilized soil conditions, respectively. Neem, tridax and achyranthes were the alternate hosts for the pathogen. Bordeaux mixture 1% spray was very effective in reducing the initial inoculum of the pathogen.

In vitro and *in vivo* evaluation of bactericides indicated that bronip (0.05%) + COC (0.2%) was highly effective in managing the disease with higher yield levels.

In biological control, *Bacillus subtilis, Pseudomonas fluorescens* and garlic extract (10%) were significantly effective in reducing the disease. Application of multinutrients (1%) recorded the lower incidence and severity of the disease. The IDM strategy evaluated was found successful and feasible in managing the disease than farmers' method of disease control.