"EPIDEMIOLOGY AND MANAGEMENT OF MANGO BACTERIAL LEAF SPOT CAUSED BY *Xanthomonas campestris* pv. *mangiferaeindicae* (Patel *et al.*) Robbs *et al.*"

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(PLANT PATHOLOGY)

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2021

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BY

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M.Sc. (Agriculture)

A thesis submitted to
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
AGRICULTURE
(PLANT PATHOLOGY)

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PARBHANI 431 402 [M.S.], INDIA

2021

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled, **"Epidemiology and management of Mango bacterial leaf spot caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (Patel *et al.*) Robbs *et al.*"**, submitted by me is based on the actual work carried out by me under the guidance and supervision of **Dr. Kalyan Tukaram Apet.** The extent of information derived from the existing literature have been duly cited and referenced. The existing research work or its any part is not submitted anywhere else for the award of any degree or diploma.

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ACKNOWLEDGEMENT

"Gratitude is a memory of the heart" I think it is matter of pleasure to glance back and recall the path during the days of hard work and study. At the last moment has to look into the deeper layers of heart, which is filled with the feelings of togetherness, loveliness and satisfaction. A number of near and dear persons to whom I acknowledge my warm regards and take this opportunity to express my feelings during the course of research. Words would probably be insufficient to express my gratitude, but words are all I have as medium of my expressions.

My diction is too poor to translate the sense of gratitude into words and heartfelt reverence and deep sense gratitude to my esteemed chairman **Dr. K. T. Apet,** Head, Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani, a person with height of human relationship, kindness and patience. I am really grateful to him for providing an atmosphere of freedom of work during course of investigation and preparation of manuscript. The thesis would not have come to a successful completion, without the help and facilities provided by him. It is because of his moral support, long lasting help and affection on me during my course of study.

I have immense pleasure in expressing my whole hearted sense of gratitude and indebtedness to Dr. A. S. Dhawan, Honorable Vice-Chancellor, VNMKV, Parbhani, Dr. D. N. Gokhale, Director of Instruction and Dean, F/A VNMKV, Parbhani, Dr. D. P. Waskar, Director of Research, VNMKV, Parbhani and Dr. D. N. Gokhale, Associate Dean and Principal (PG), COA, Parbhani, VNMKV, Parbhani for providing necessary facilities during the entire course of study my research work.

I am equally grateful to other members of my advisory committee Dr. D. N. Dhutraj, Associate Dean and Instruction, VNMKV, Parbhani, Dr. P. R. Zanwar, Associate

Professor, Department of Agril. Entomology, VNMKV, Parbhani Dr. S. J. Shinde, Associate Professor, Department of Horticulture, COA Golegaon, VNMKV, Parbhani and Dr. D. G. Dalvi, Superintendent Agril. School, VNMKV, Parbhani.

My deeply gratitude to Dr. K. T. Apet, Head, Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani, Dr. A. P. Suryawanshi, Associate Dean and Principal, COA, Latur, Dr. K. D. Navgire, Associate Professor, Dr. C. V. Ambadkar, Assistant Professor, Dr. A. T. Daunde, Assistant Professor, Dr. Minakshi Patil, Assistant Professor, Dr. S. L. Badgujar, Associate Professor, Dr. V. M. Gholve, Associate Professor, Dr. M. S. Dadke, Associate Professor, Mr. Kapil Nirwal, Agril. Assistant, Prof. P. L. Sonttake, Prof. V. Goverdhan Rao, Assistant Professor, Prof. P. B. Devre, Prof. S.V. Waghmare for their positive backing and kind cooperation during the period of investigation.

I am particularly deeply indebted to Dr. H. V. Kalpande, Associate Professor, Department of Genetics and Plant Breeding, VNMKV, Parbhani, Dr. A. M. Bhosale, Assistant Professor, Department of Horticulture, VNMKV, Parbhani and Dr. P. R. Zanwar, Associate Professor, Department of Agril. Entomology, VNMKV, Parbhani, Dr. S. K. Patil, Associate Professor, Department of Agril. Entomology, VNMKV, Parbhani for positive backing, co-operation and valuable suggestions during the course of this study.

I express my sincere thanks to the Prof. R. S. Borade, Incharge, Central nursery Scheme, VNMKV, Parbhani, for their valuable suggestions and guidance in carrying out the research. Also express my sincere thanks to Mr. Sandeep Kale, Quencher Biotech Private Limited, Pune, for his expertise and cooperation in molecular studies during the course of investigation.

I shall fail in my duties if I do not record my sincere thanks to the respondents of farmers for providing me reliable and all type of data required for the present

investigation. I am deeply obliged to all the authors and research scholars past and present, whose literature have been cited. I express my sincere thanks to librarian, Dr. S. D. Kadam, Librian, VNMKV, Parbhani for excavating literature from library and for his kind co-operation during my research work.

Everything in my life is nothing if I ignore the all time encouragement, evergreen affection and latent love of my Father Shri. Govindrao Bhagwan Patil and Mother Sau. Lata Govindrao Patil which elevated my morals during the whole educational carrier. The words fail to express the depth of humble respect and heartfelt reverence about my husband Dr. R. C. Agale for encouragement during study. I express my profound sense of gratitude, indebtedness, and hearty thanks to my uncle Dr. Sanjay Patil, brother Abhijit Patil and Nikhil Patil. Words are insufficient to take account of encouraging and evergreen love of my sweetest sister Supriya. Special thanks to Sau. Anita Apet Madam for their valuable support.

My sincere thanks to my seniors Padmavati Khandare, Pratiksha Gaikwad, Kalim Sayyad and my juniors Navya Tejaswini, Amruta Karnewar, Krishnaveni, Asha Kendre, Deepali, Vaishali Karnale, Akshay Ingole, Ram Raner, Samadhan Pawar, Ganesh Pawar, Nayan.

Also my classmates Namdeo Dhurve, Rahul Dake, Sandip Aher, Sarika Timke, Shabnam Didi, Nilima Didi, Dhanshree Didi, Savita Didi. Friendship is pleasant experience most at all, my words and very special thanks to my friends Shanmukhi and Navya for their good will and moral support. And my M. Sc. batchments Vishal Kashid, Dnyaneshwar Bangar, Sandhya Limkar, Chetna Kumbhar, Sonam Vaishnav and Sayli Salunkhe.

I am especially grateful to Nitin kakde, Laxman mama, Kadam mama, Chapate mama, Maske Mama, Manik Londe, Hashina Khalla, Shinde Maushi and Mohite Maushi

for their kind cooperation and help during the course of this work and carried out the difficult task of everything only swiftly and accurately but cheerfully.

Last but never least, it is difficult to list all those to who express my gratitude for their goodwill and moral support during my academic attainment, through nobody is mentioned none is forgotten too. Above all I bow my head before almighty God whose constant blessings enabled me to complete this research work successfully and give me courage to stand all.

I am very grateful to Chhatrapati Shahu Maharaj Research, Training and Human Development Institute (SARTHI) to give me financial assistance under CMSRF-2019 (Chief Minister Special Research Fellowship- 2019) to be paid by SARTHI to my higher studies for Ph.D. Agriculture, directly to my saving bank account.

CMSRF-2019 fellowship directly help me foe various aspects, viz., purchase of chemical insecticides, research books, publication of my research article, implementation of research methodology which are paid (can't possible without CMSRF-2019 fellowship to students), printing and binding of research paper, research article and Ph.D. thesis. These all activities I do because SARTHI CMSRF-2019 fellowship.

This fellowship is very important in view of Ph.D. student and I personally request to SARTHI, keep it on in future which helps increase level of education, research and development in our student that resulted great reflection on our state and our country. Thank you SARTHI once again to award me CMSRF-2019 fellowship.

In advertently I might have forgotten to mention the names of invisible hands for their help, I am thankful to them as well.

A G Patil

(A. G. Patil)

Reg. No. 2017A/14P

Place : Parbhani

Date : 24/05/2021

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ABBREVIATIONS USED

%	=	Per cent
/	=	Per
@	=	At the rate of
µg	=	Microgram
BOD	=	Biological Oxygen Demand
bp	=	Base pair
C.D.	=	Critical Difference
Cm	=	centimeter (s)
CRD	=	Completely Randomized Design
cv.	=	Cultivar
DAS	=	Days after sowing
Dia.	=	Diameter
DNA	=	De-oxy ribonucleic acid
dNTP	=	Deoxyribose Nucleotide Tri-Phosphate
e.g.	=	Exempli gratia (for Example)
EDTA	=	Ethylene Diamine Tetra Acetic Acid
et al.	=	and others
EtBr	=	Ethidium bromide
etc.	=	Etcetera
Fig.	=	Figure (s)
G	=	Gram
Ha	=	Hectare (s)
hrs.	=	Hours
i.e.	=	That is
kb	=	Kilobase pair
Kg	=	Kilogram (s)
L	=	Litre
M	=	meter (s)

Max.	=	Maximum
Mg	=	Milligram
$MgCl_2$	=	Magnesium chloride
Min.	=	Minimum
Ml	=	Milliliter
Mm	=	millimetre
mM	=	Millimolar
NaCl	=	Sodium chloride
ng	=	Nanogram
No.	=	Number (s)
^0C	=	degree Celsius
OD	=	Optical density
PCR	=	Polymerase chain reaction
PDA	=	Potato Dextrose Agar
PDB	=	Potato Dextrose Broth
PDC	=	Per cent Disease Control
pH	=	Log H+ ion concentration
pmol	=	Picomole
Psi	=	pounds per square inch
q/ha	=	quintal per hectare
RAPD	=	Randomly Amplified Polymorphic DNA
RBD	=	Randomized Block Design
RNA	=	Ribonucleic acid
RNase	=	Ribonuclease
rpm	=	revolutions per minute
SDS	=	Sodium Dodecyl Sulphate
Sec.	=	Seconds
SEm	=	Standard error of mean
sp.	=	Species
T	=	Treatment
t/ha	=	tonnes per hectare

TAE	=	Tris acetate EDTA buffer
Taq	=	Thermus aqaticus
Tris HCL	=	Tris Hydrochloride
viz.,	=	videlicet (namely)
wt.	=	Weight

THESIS ABSTRACT

Epidemiology and management of Mango bacterial leaf spot caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (Patel *et al.*) Robbs *et al.*

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THESIS ABSTRACT

Mango (*Mangifera indica*) is the national fruit of India. Mango is cultivated in most frost free tropical and warmer subtropical climates in the world. Mango bacterial leaf spot, (*X. campestris* pv. *mangiferaeindicae)* is one of the most devastating diseases throughout the world, affecting all kinds of commercial mango varieties (Gagnevin and Pruvost, 2001). Therefore, present investigation on bacterial leaf spot of mango caused by *X. campestris* pv. *mangiferaeindicae* with the objectives *viz.,* collection of diseased samples, pathogenicity, morphocultural, biochemical, pathogenic and molecular variability amongst *X. campestris* pv. *Mangiferaeindicae* isolates, epidemiological studies, *in vitro* efficacy of antibiotics, antibacterial fungicides plant essential oils and bioagents were undertaken during 2017-2018 to 2019-2020 at VNMKV, Parbhani (M.S.), India.

Studies conducted during sample collection on disease intensity indicated that, bacterial leaf spot of mango is of common occurrence in all agro-climatic zones of Marathwada region of Maharashtra state exhibiting different per cent intensity of bacterial leaf spot on mango plants. However, maximum per cent intensity was recorded in Anandwadi (45.30%) from Scarcity zone whereas; minimum per cent intensity was recorded in Kandhar (20.11%) from assured rainfall zone.

The pathogen *X. campestris* pv. *mangiferaeindicae* was successfully isolated on NA media from naturally bacterial leaf spot diseased leaf, fruit and twig specimens of mango collected and about eight isolates, one each representative of eight districts of Marathwada region were obtained, purified and used. Pathogenicity of all test isolates was successfully proved on mango cultivars under screen house as well as under lab conditions. Typical symptoms were observed on the leaves and

fruits, initially small, water soaked lesions delineated by veins were noticed on lower surface of the leaves followed by appearance of small brown to dark brown coloured spots on the upper surface with circular to irregular shape with chlorotic halo. On developing green fruits, symptoms were noticed as small pin head sized, black lesions with diffused water soaked margin, which later developed into black coloured, medium to big sized erumpent cankerous.

Cultural, morphological, symptomatic and molecular studies revealed exhibited a wide range of which variability of among eight isolates of *X. campestris* pv. *mangiferaeindicae* which represented three agro-climatic zones of the Maharashtra.

Studies on detached leaf technique under controlled lab conditions revealed that, leaves of total six varieties of mango viz., Local, Kesar, Dasheri, Neelam, Amrapali and Alphonso were used and observation were recorded on incubation period and symptom type. The entire eight test isolates of *X. campestris* pv. *mangiferaeindicae* found pathogenic and caused bacterial leaf spot in leaves of all mango cultivars.

The result of host range revealed that symptoms of the disease were not observed in all the tested fruit crops. The bacterium is a potential threat to infect the mango crop. Effect of different age of leaf on disease development (On detached leaves) showed that old age leaves were more susceptible than mid age and young age leaves.

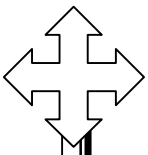
Correlation between disease and weather factors on fruit revealed that, maximum temperature had significant positive correlation with the disease development in both the years. Role of minimum temperature was significant and positive with the disease development. Relative humidity had negative significant relationship with the disease in both the years while, rainfall had positive significant relationship in the year 2018 and negative significant relationship in the year 2019 with the disease. Correlation between disease and weather factors on leaves revealed that, maximum temperature had significant positive correlation with the disease development in both the years. Role of minimum temperature was significant and positive with the disease development. Relative humidity had positive significant

relationship with the disease in both the years. Rainfall had positive significant relationship with the disease development in both the years.

Stepwise regression models showed strong and positive relationship between combined effects of weather factors for disease development. The apparent infection rate of the disease was slow initially, which gradually increased when temperature and humidity became favorable in both the years. The area under disease progress curve (AUDPC) was more on leaves as compared to fruits in both the years.

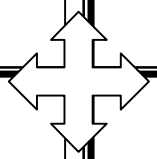
In vitro studies on antibiotics, antibacterial fungicide, plant essential oils and bioagents revealed of all antibiotics tested, Streptocycline was found most superior with significantly highest (15.30 mm) average inhibition zone, amongst antibacterial fungicides: Copper oxychloride 50% WP found most superior with significantly highest (12.98 mm) average inhibition zone while, Cinnamon oil was found most superior with significantly highest (14.03 mm) inhibition zone. However, of all the bioagents tested, significantly highest inhibition of test pathogen was found with *P. fluorescens* (15.30 mm).

All antibiotics, fungicides, essential oils and bioagents tested (pot culture) against *X. campestris* pv. *mangiferaeindicae* significantly reduced the disease intensity after first and second spraying over control. However, highest mean per cent disease control of was recorded with Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w (44.01 %), followed by Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (34.57 %); whereas, it was minimum with *P. striata* (21.13 %).



CHAPTER-I

INTRODUCTION



CHAPTER - I

INTRODUCTION

Mango (*Mangifera indica*) is cultivated in most frost free tropical and warmer subtropical climate. Tree is sympodially branched, medium to large, evergreen with symmetrically rounded canopy ranging from low and dense to upright and open. The inflorescence is pseudo-terminal originated from a bud with hermaphrodite and male flowers in same panicle. Fruit is a more or less compressed fleshy drupe (Anonymous, 2019).

Mango belongs to family Anacardiaceae. It is the national fruit of India. Beside delicious taste, excellent flavour and attractive fragrance, it contains a variety of nutrients and rich in vitamin A & C. The energy value per 100 g serving of common mango is 60 kcal. The major mango growing states are Uttar Pradesh, Andhra Pradesh, Bihar, Karnataka, Kerala, Tamil Nadu and Maharashtra. Uttar Pradesh ranks first in the mango production with the share of 23.47 % and highest productivity (APEDA, 2019).

The centre of origin and diversity of the genus *Mangifera* is firmly established in Southeast Asia. Almost half of the world's mangoes are cultivated in India alone with China and Thailand is the next largest producers. In 2019, global production of mangoes was 55.4 million tonnes led by India with 39 % (22 million tonnes). Mangoes are also grown in Andalusia, Spain, South and Central America, the Caribbean, Hawaii, South, West and Central Africa, Australia, China, South Korea, Pakistan, Bangladesh and Southeast Asia.

India ranks first in the production, consumption and export of mango all over the world with an area, production and productivity of 2262.8 000' ha, 19686.9 000' MT and 8.7 MT/ha respectively whereas, Maharashtra occupies an area of 157.07 ha, production 520.87 t and productivity of 3.58 MT/ha (Anonymous, 2019).

Mango is affected by a number of diseases from the seedling stage to the fruits in the storage or transit. Bacterial leaf spot, anthracnose, black tip, powdery mildew, sooty mould and phoma blight are of major concern of growers in India (Omprakash, 2004).

Mango tree is prone to attacks by a number of pests and diseases, certain types of pests may kill the tree but most would debilitate, which causes heavy losses in the field and during storage and also result in severe losses (Akhtar and Alam, 2002). The number of diseases affecting mango can seriously limit the production, if not adequately controlled (Pernezny and Ploetz, 2002).

Mango bacterial leaf spot disease which is also known as mango canker, bacterial spot, bacterial canker, black spot, mango blight, bacterial black spot is caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (*Xcmi*) (Gupta and Sharma, 2001). It is one of the most destructive bacterial disease of Mango worldwide (Gagnevin and Pruvost, 2001). It was first reported by Patel *et al*., (1948) from Pune and Dharwad as bacterial black spot. The disease is also prevalent in Africa (Doidge, 1915; Steyn *et al*., 1974), Pakistan (Khan and Kamal, 1966), Brazil (Robbs *et al.,* 1974) and from many other mango producing countries (Gagnevin and Pruvost, 2001). During summer months the disease appears in most serious forms in many production areas of India (Kishun, 1982). It was recently recorded in different regions of Hormozgan province of Iran for the first time (Najafipour, 2014).

Many commercial cultivars are highly susceptible to bacterial leaf spot and infections can result in drastic yield losses associated with premature fruit drop, reduction of fruit quality and induction of severe defoliation especially when storms or hurricanes are involved. About 50 to 80 % fruit infection is common on very susceptible cultivars. Mango bacterial leaf spot epidemics occurred in most of the mango producing areas of South Africa, causing almost 100 % fruit loss (Gagnevin and Pruvost, 2001). High crop losses (more than 80 %) can occur, especially after a cyclonic depression causing high winds and heavy rains (Aubert, 1981). More than 50 % of crop losses reported due to this disease in some orchards of peninsular Malaysia (Lim *et al*., 1991). The pathogen causes heavy losses to crop under favourable environmental conditions (Shekhawat and Patel, 1975).

The bacterial canker incited by *Xanthomonas campestris* pv. *mangiferaeindicae* is an endemic problem in the major mango growing regions of the world *i.e*., Asia, Southern and Eastern Africa, Western Oceania and Indian Ocean (CPC, 2005). Since last more 2 decades the disease became important in India (Kishun, 1995). The pathogen affects all the above ground plant parts and results in

substantial loss in fruit yield and quality (Shekhawat and Patel, 1975; Kishun, 1981). Lesions on leaves are angular, raised, black and necrotic, whereas on fruits these are star shaped and erumpent with infectious gummy exudates. Occasionally, twig cankers can develop (Pruvost and Manicom, 1993). Bacterium survives mostly in lesions and on arial parts of mango (Pruvost and Chand, 1994). The pathogen is believed to spread between continents or countries through contaminated planting material and on a smaller scale, by cultural practices and wind splashed rain (Manicom, 1986).

Early stage of fruit and leaf lesions appears as small necrotic lesions (2-3 mm in diameter) with surrounding water soaked area (Chand and Kishun, 1995; Sanders *et al*., 1994). Mature symptoms on leaves are small angular, black and raised, releasing gum. Bacteria enter the plant through wounds, stomata or lenticels (Gagnevin *et al*., 1997). On fruit, lesions develop around lenticels or wounds and are irregular in shape (Manicom, 1992). Lesions eventually dry out; turn black and crack open (Manicom and Frean, 1992). Raised blacked spots with greasy margins can also later develop on leaves. Stem lesions often appear, as blackened cankers crack longitudinally, also releasing gum. Disease incidence is high when rainfall with strong winds and high temperatures occur at the same time (Manicom, 1986). Disease incidence is also greater on exposed and abraded leaf and fruit surfaces or where fruits touch each other (Pruvost and Luisetti, 1991). The Pathogen survives mostly in lesions and on aerial organs as epiphytes and enters the plant through plant wounds, stomata and lenticels (Pruvost *et al*., 1993). The pathogen can also be transmitted through irrigation water, insects and mechanical transfer of infected planting material (Pruvost and Manicom, 1993).

The average population of the bacterium on mango tree surfaces can as high as 10^6 cfu per leaf or fruit. Surface population varies with climatic conditions is correlated with the number of lesions per leaf of fruit. Humid conditions favour the epiphytic growth. The presence of free water allows the release and redistribution of bacteria from the ruptured epidermis. The population quickly decreases to undectable levels during dry conditions. Populations of 10^{4-6} cfu per fruit can be found on young fruits under natural conditions and probably these constitute inoculum associated with latent infection, which will be responsible for symptoms when the fruit receptivity increases.

Considering these facts, importance of crop and disease, present studies were planned and conducted on bacterial leaf spot of mango caused by *Xanthomonas campestris* pv. *mangiferaeindicae* with the following objectives:

OBJECTIVES :

1. To collect bacterial spot diseased plant samples of mango from different agroclimatic zones of Marathwada region.
2. To isolate, characterize and prove the pathogenicity of *Xanthomonas campestris* pv. *Mangiferaeindicae* isolates.
3. To study variability *viz*., pathogenic, morpho-cultural, biochemical and molecular among isolates of *Xanthomonas campestric* pv. *mangiferaeindicae*.
4. Epidemiological studies of *Xanthomonas campestris* pv. *mangiferaeindicae*.
5. To evaluate *in vitro* efficacy of antibiotics, antibacterial fungicides and bioagents against *Xanthomonas campestris* pv. *mangiferaeindicae*.
6. To evaluate *in vitro* efficacy of essential oils against *Xanthomonas campestris* pv. *mangiferaeindicae*.

CHAPTER-II

REVIEW OF LITERATURE

CHAPTER -II

REVIEW OF LITERATURE

The important literature pertaining to *Xanthomonas campestris* pv. *mangiferaeindicae* and other *Xanthomonas* spp. on the different aspects is reviewed under the following heads.

2.1. Occurrence, distribution and yield losses

2.2. Symptomatology

2.3. Isolation and Identification of *Xanthomonas* spp.

2.4. Variability among *Xanthomonas* spp.

- 2.4.1. Pathogenic variability
- 2.4.2. Morpho-cultural characters
- 2.4.3. Biochemical characters
- 2.4.4. Molecular variability
- 2.4.5. Host range

2.5. Epidemiological studies

- 2.5.1. Role of Weather Factors

2.6. Disease management strategies

- 2.6.1. Bioefficacy of antibiotics and chemicals
- 2.6.2. Bioefficacy of bioagents
- 2.6.3. Bioefficacy of plant essential oils

2.1. Occurrence, distribution and yield losses

The bacterial blight disease was first reported by Doidge in 1915 from the South Africa. Since then it was reported from different parts and countries like Australia, the Combo Islands, Japan Malaysia, Mauritius, New California, Pakistan, the Philipines, Reunion Island, Taiwan, Thailand and United Arab Emirates. The disease was also reported from most of the parts of Asia and eastern parts Africa (Gagnevin and Pruvost 2001). Patel *et al*. (1948) for the first time reported bacterial leaf spot of mango from India and named the pathogen as *Pseudomonas*

mangiferaeindicae. The disease was prevalent in Maharashtra and Madras Province and probably it existed since 1881 in Bihar and several varieties of mango are susceptible for disease.

Shekhawat and Patel (1975) observed bacterial leaf spot of mango disease in Delhi, Uttar Pradesh and Haryana on Langra variety, affecting 10-85 per cent fruits of mango. Maximum fruit infection was observed in the Institute's orchard hybrids (10-100 %) followed by Bangalora (40 %), Neelum (5 %) and Langra (2 %). Out of all diseased fruits 20-26 per cent developed rotting before harvest and 20-47 per cent during ripening period.

Kisun (1982) reported the dropping of mango fruit due this pathogen in Alphanso (20-80 %), Payri (10-70 %), Totapuri (10-40 %) and local verities (10-55 %). The maximum fruit drop was after 30 -60 days of fruit set. Also he reported that pathogen was responsible for fruit rot in storage.

Lim *et al*. (1991) reported that more than 50% of crop losses due to black spot of mango occurred in some orchards of peninsular Malaysia during the months of February-March, September-November in the year 1988.

Chand and Kishun (1995) developed SXTPA, a semi-selective medium for the isolation of the bacterium causing bacterial leaf spot of mango.

Khan and Mirza (1995) reported bacterial leaf spot of mango from the samples collected from the orchards of Faisalabad, Pakistan. The isolated bacterial strain was confirmed as *Xanthomonas campestris* pv. *mangiferaeindicae.* The isolated strain caused leaf spot which later developed into canker.

Gagnevin and Pruvost (2001) reported the disease from Australia, the Comoro Islands, Mauritius, Pakistan, Philippines, India, Japan, Kenya, Malaysia, Africa and Thailand. They also reported that the disease caused 100 per cent fruit loss in South Africa in the year 1996 and 1997.

Haggag (2010) observed the canker disease of mango, which was caused by a bacterium (*Xanthomonas campestris pv. mangiferaeindicae*), prevalent in

Egypt. The disease caused fruit drop (10-70%), yield loss (10-85%) and storage rot (5-100%).

Pruvost *et al.* (2011) stated that the mango bacterial canker pathogen severely affects most commercial cultivars in the old world and was a serious threat for industries in Central and South America, in countries like Mexico, Brazil, and Peru ranked second, third, and fourth, respectively, in 2007 world trade). He also stated that the favouring, the pathogen can cause fruit losses up to 85%.

Thirumalesh *et al*. (2012) observed more than 80% crop losses in fruits of mango caused due to bacterial leaf spot and also they reported the bacterial spots of leaves on different cultivars grown in Kolar and Chitradurga districts of Karnataka.

Najafipour *et al*. (2014) reported that bacterial black spot of mango caused by *Xanthomonas citri* pv. *mangiferaeindicae* for the first time in Hormozgan province of Iran.

Sanahuja *et al*. (2015) observed symptoms for the first time on mature fruits of mango in the vicinity of Boynton Beach and Lake Worth of America where environmental conditions in Southern Florida appear to be ideal for an increased importance of bacterial canker in the region as well as other areas in the Americas in which production of mango occurs.

Zombre *et al.* (2016) observed mango leaves showing typical symptoms of bacterial leaf spot caused by *Xanthomonas campestris* pv. *mangiferaeindicae* for the first time in five regions of Togo such as Savanes, Kara, Centrale, Plateaux and Maritime. They reported high disease prevalence was observed in three Northern provinces *i.e* Savanes, Kara and Centrale.

Honger (2018) reported that mango bacterial black spot as a devastating disease which can cause yield losses up to 100% since the disease could cause heavy dropping of immature fruits and could leave rotten dark spots on fruits that go into maturity. Therefore, in severe infection, may all fruits dropped.

2.2. Symptomotology

Gagnevin and Pruvost (2001) described leaf and fruit symptoms were most common but twig and branch cankers occurred when infection was severe. Leaf symptoms began as small, water soaked spots delineated by veins, becoming raised, black, sometimes with a chlorotic halo. Fruit symptoms appeared as small water soaked spots on lenticels and they later became star shaped, erumpent, and exuded an infectious gum.

Akhtar and Alam (2002) reported that the infected leaves produced angular, water soaked spots of 1-3mm in diameter, which were delimited by the veins.Those coalesced, became black and slightly raised and exuded gum under very humid conditions. Older lesions turned light gray, dry out and crack. Stem lesions appeared as blackened cankers that formed longitudinal cracks with bacterial exude. Fruit lesions developed halos around lenticels or wounds and soon became raised and then blackened and cracked open with gummy infection.

Stovold (2004) described bacterial blight diseases in mangoes and bacterial black spot of mango is a bacterial disease caused by *Xanthomonas campestris* pv. *mangiferaeindica* unlike Anthracnose, bacterial lesions do not expand as the fruit ripen. The disease infects through natural openings such as stomata, wax and oil glands, leaf and fruit abrasions, leaf scars and at the apex of branches in the panicle in young trees the diseases can cause dieback of branches.

Pitkethley (2006) stated that bacterium that caused black spot was called *Xanthomonas campestris* pv. *mangiferaeindicae* it can attack leaves, twigs and fruit the main symptoms of leaf spots are black and raised, they tend to angular in shape and confined by larger veins, black scabby spots are formed on fruit often with star shaped cracks within them. The spots have water soaked margins.

Ah-You *et al*. (2007) described symptoms of bacterial leaf spot on leaves of mango as raised, black, angular and necrotic and those on fruits were star shaped, erumpent with gummy exudates. Lesions on twigs or branches occasionally occurred, most often after hurricanes, but no twig dieback was reported. They also reported brownish, flat, angular spots from Brazil and also induced unique twig

symptoms *i.e.,* yellow to brown lesions, which were subsequently longitudinally cracked and evolved as a generalized dieback of the youngest vegetative flush.

Jackson (2009) stated that symptoms of mango bacterial black spot disease could be found in all the above-ground parts of the mango tree. On leaves, symptoms begin as small water-soaked spots delineated by veins, becoming raised, black, sometimes with a chlorotic halo.

Haggag (2010) described that bacterial leaf spot of mango and noticed angular, water soaked spots or lesions, surrounded by clear holes on leaves which became necrotic dark brown and viscous bacterial exudates deposited on those necrotic portions that became corky and hard after drying. Sometimes, longitudinal cracks were also developed on the petioles and cankerous lesions appeared on petioles, twigs and young fruits. Water soaked lesions developed on fruits became brown to black.

QI,Y *et al.* (2012) observed symptoms characterized by small water soaked spots on the leaves which turned into angular, sunken, deep brown to black necrotic lesions with a yellow halo with age, whose necrotic tissue fell resulted in shot hole condition of the blades.

Thirumalesh (2012) reported typical bacterial leaf spot symptoms on leaves, twigs, branches and fruits of mango collected from various regions of Karnataka. Symptoms characterized by small, water soaked lesions on leaves that subsequently turned into dark brown to black spots whereas, branches and twigs with discoloured areas at the nodes that turned brown or necrotic were observed. Fruits with discoloured, depressed, water soaked black spots or with lesions, cankers were observed.

Yasuhara *et al*. (2012) observed black lesions on mango fruits and on leaves black lesions with yellow halos in mango trees infected with bacterial leaf spot from Hawaii.

Thind (2012) described the symptoms of mango bacterial leaf spot appeared on the aerial parts of the mango tree. Leaf and fruit symptoms are most common but symptoms on twigs and branches occur on highly susceptible cultivar.

On leaves, symptoms appear as minute, water-soaked, irregular spots, which enlarge, turn brown or dark brown, become angular due to delineation by veins and finally cankerous and raised. Most of the lesions are surrounded by chlorotic haloes. On fruits, symptoms appear as small, water-soaked spots on lenticels. These spots later become star-shaped, develop into cankers, which are much more raised and darker than those on leaves, and often burst open releasing a highly contagious gummy substance containing bacterial cells. On twigs and branches, the lesions are raised having longitudinal rifts, predominantly formed on the under surface but without any gummy ooze. Several lesions may run together forming longitudinal scars.

Khatua *et al*. (2013) stated that symptoms of mango bacterial leaf spot were recorded on almost all aerial parts of the plant including leaves, petioles, fruits, flower stalks, tender stems and branches.

Najafipour *et al*. (2014) described that leaves affected with mango bacterial black spot showed brown to dark, flat and angular spots.

Sanahuja *et al*. (2016) reported that fruits of mango infected with bacterial leaf spot showed star shaped lesions up to 2cm in diameter erupted from the surfaces and oozed sticky clear exudates.

Zombre *et al.* (2016) reported that mango leaves showed typical symptoms of bacterial leaf spot, lesions were black, slightly raised, angular and sometimes contained a chlorotic halo. Later in the season, fruit symptoms consisted of small water soaked spots around lenticels that later developed into black star shaped erumpent lesions. Moreover, twig cankers were also observed sporadically.

Irfan *et al.* (2017) observed mango bacterial black spot was characterized by lesions development on leaves, twigs and fruits. Leaf lesions were black, raised and tend to be angular in shape as they were confined by the large veins frequently surrounded by yellow margins. Bacterial lesions do not expand with fruit ripening. Old lesions dry and turn brown ash-gray. Twig and stem lesions were black and cracked. Spots on fruit were scabby black often with star shaped cracks and water soaked margins.

Bandi (2019) observed the mango bacterial leaf spot symptoms as, small water soaked lesions delineated by veins on the lower surface of leaves followed by appearance of small brown to black coloured spots on the upper surface with round to irregular shape with chlorotic halo. As the disease progressed, the spots also grew, increased in size, coalesced and developing green fruits, symptoms were noticed as small, pin head sized, black lesions with diffused water soaked margin, which later developed into black coloured, medium to big sized erumpent cankerous spots with some times showing characteristic tear staining symptoms.

2.3. Isolation and Identification:

Patel *et al*. (1948) first described the bacterium and named it *Pseudomonas mangiferae-indicae* sp. nov.

Robbs *et al*. (1974) reclassified the bacterium as *X. campestris* subsp. *mangiferae-indicae* sp. nov. With the introduction of pathovar system for the classification of plant pathogenic bacteria, it was changed to *X. axonopodis* pv. *mangiferae-indicae*

Moffett *et al*. (1977) isolated bacterial black spot causing pathogen using sucrose peptone agar and pathogenicity was established by spray inoculating young mango leaves with a suspension of bacterium in sterile water.

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

Manicom and Wallis (1984) isolated strains of bacteria causing bacterial leaf spot of mango using nutrient agar medium and proved pathogenicities of strains by pressure infiltrating suspensions containing 10^6 colony forming units per ml into the leaves of *M. indica* plants.

Sanders and Korsten (1995) described a rapid method for the differentiation of epiphytic *Xanthomonas campestris* pv. *mangiferaeindicae* where

boost broth supplemented with cycloheximide, methyl violet and methyl green (BVGA medium) enhanced the growth of this bacterium. The identity of *xcmi* was confirmed with monoclonal antibodies in an enzyme linked immuno-sorbent assay.

Dayakar and Gnanamanickam (1996) isolated *X. campestris* pv. *mangiferaeindicae* using yeast nutrient agar (YNA) medium, from infected plant parts such as leaf, inflorescence axis, petiole and fruit of mango and obtained fluidal, white single colonies that appeared after 48-72 h at 28˚C were picked up and again streaked onto YNA medium for obtaining pure cultures. They proved pathogenicity by rubbing inoculums on mango leaves with the help of sterile cotton.

Manjula (2002) obtained the pure culture of the seven isolates of the *Xanthomonas axonopodis* pv. *punicae* from infected leaf, twig and fruit samples of pomegranate collected from different locations, on nutrient agar medium, by dilution planting technique and proved their pathogenicity by spray inoculation method, on variety Jyoti.

Pruvost *et al.* (2005) developed the two semi-selective media for the isolation of mango bacterium, namely, KC and NCMT3 having plating efficiencies ranging from 76-104% and from 78-132% respectively. In the later case, the repeated isolations of the pathogen were possible even from symptomatic leaves.

Ah-You *et al* (2007) changed the name of *Xanthomonas campestris* pv. *mangiferaeindicae* to *Xanthomonas axonopodis* pv. *Mangiferaeindicae* and finally to *Xanthomonas citri* pv. *Mangiferaeindicae* in the year 2009.

Didwania *et al.* (2013) isolated black rot causing bacterium *Xanthomonas campestris* pv. *campestris* from naturally infected cauliflower leaves showing typical 'V' shaped symptoms. Single fresh and pure glistering yellow droplets like colonies were purified and maintained on nutrient agar medium.

Arshadi *et al*. (2013) collected citrus canker diseased samples from citrus trees and isolated on NA medium. Following incubation, colonies similar to *Xanthomonas* were subcultured on YDC semi-selective medium.

Al-Saleh *et al.* (2014) isolated 76 *Xanthomonas* strains from different citrus species showing bacterial canker symptoms. Bacterial colonies with yellow pigmentation were transferred on to NGA plates.

Pawar *et al.* (2014) isolated bacterial colonies from fruits of pomegranate infected with *Xanthomonas axonopodis* pv. *punicae* on Starr's agar medium. After the incubation period observations were made for the development of well separated, typical, light yellow coloured bacterial colonies resembling *Xanthomonas* sp. also proved the pathogenicity by Pin-Prick method on six month old pomegranate seedlings.

Pawar (2014) isolated *Xanthomonas campestris* pv. *vesicatoria* causing bacterial spot of tomato using nutrient agar medium (NA) by streaking and proved pathogenicity by adopting koch's postulates.

Rashid *et al*. (2016) isolated bacterial leaf spot pathogen from mango leaves by dilution plate technique. Modified nutrient glucose medium (glucose= 5g/L and nutrient agar = 15g/L) was used for bacterial colony isolation and recovered colonies were purified by streaking method.

Ofoe *et al*. (2016) isolated *Xanthomonas campestris* pv. *mangiferaeindicae* from diseased leaves branches using nutrient agar medium and was then sub-cultured twice on nutrient agar to obtain single colony forming units (cfu) of the bacterium and also proved pathogenicity by creating artificial wounds on leaves of mango seedlings.

Sanahuja *et al.* (2016) isolated *Xanthomonas campestris* pv. *mangiferaeindicae* on yeast peptone glucose agar medium and proved pathogenicity of strains by infiltration method where mango varieties Kiett and Haden plants were injected with 18 hour old YGPA colonies (1 x 10^5 CFU/ml). After 7 days, black lesions developed on inoculated leaves.

Katkar *et al.* (2016) collected symptomatic samples of citrus canker from the 14 Agro-climatics region of the India and the different plant parts like infected leaves, twigs and fruits were used for isolation of pathogen by tissue isolation method. The isolation of *Xanthomonas axonopodis* pv. *citri* was done on Nutrient

Agar (NA) medium. The typical bacterial colonies showing characteristics of *Xanthomonas spp.* were maintained on the slant containing Yeast Extract Glucose Chalk Agar (YGCA) medium and subsequently sub cultured at regular intervals.

Bandi (2019) isolated *Xanthomonas campestris* pv. *mangiferaeindicae* from the infected leaf and fruit parts of mango plant by following the streak plate technique using nutrient agar medium. The colonies appeared after four two six days interval of time after the incubation. Culture of each isolate was purified by streaking suspected single colony on nutrient agar medium and maintained on slants.

2.4. Variability among *Xanthomonas* spp.

2.4.1. Pathogenic variability

Tsuchiya *et al.* (2003) isolated *Xanthomonas campestris* pv. *mangiferaeindicae* strains on YPA solid medium. They tested pathogenicity using 2-year old plants and on detached leaves of mango. The tests were done on whole plants using an atomizer where young green twigs and mature green leaves were wounded with needles and sprayed with inoculums prepared by standardizing cell suspensions about 10^8 cfu/ml. The plants were covered with polyethylene bags and kept in greenhouse at 20˚- 28˚C under natural light. Detached leaves were aseptically inoculated with 50 µl inoculums (10^8 cfu/ml) and were placed at 25˚C under fluorescent light. Symptom development was observed after a week of inoculation.

Sain *et al.* (2008) studied pathogenic variability among 12 isolates of *Xanthomonas campestris* pv. *campestris* causing black rot of cauliflower collected from different locations of vegetable farm at IARI, Pusa and surrounding regions of Delhi on different ten cultivars of cauliflower using radiant streak plate method. They reported that 6 isolates *viz.,* Xc-2, Xc-4, Xc-6, Xc-7, Xc-10, Xc-12 were only mild to moderate infections on Pusa synthetic and CC-12, other cultivars (P-522, HR-6-1, PSR-1, Se1-9, Lawanya, HR-5-1, BR-1, Sel-9-1) were more or less free from any infection.

Meena (2010) collected 14 citrus canker diseased samples from various places of Marathwada region and isolated the bacterium (*X.axonopodis* pv. *citri)* on nutrient agar by streak plate method. The bacterial colonies developed were

circular, convex, mucoid, shiny and yellow. The pathogenicity test resulted with typical symptoms of citrus canker, within 10 days after inoculation.

Kale *et al.* (2012) studied the isolate of *Xanthomonas axonopodis* pv. *punicae* causing oily spot of pomegranate collected from Nashik district for its pathogenicity on one year pomegranate plant by two method *viz.,* Infiltration by syringe and surface spreading by painting brush method. They also categorized symptoms developed on plant after 5, 10, 15 and 20 days after inoculation has single pinheaded water soaked spot, (+++) brown and oily angular spot surrounded by water soaked ring, (++++) black water soaked spot surrounded by yellow ring on leaves, (+++++) yellow leaves with black oily spot ready to senescence and (-) no symptoms observed. Further they reported that typical symptoms were observed within a week on the leaves; while the development of symptoms in case of in filtered leaves was earlier than uninjured surface inoculated leaves.

Thirumalesh *et al.* (2012) isolated and identified four strains of *X.c* pv. *mangiferaeindicae* and strains were cultured on yeast extract nutrient agar(YNA) medium and proved pathogenicity by spray inoculation technique.

Yasuhara *et al*. (2012) isolated *Xanthomonas campestris* pv. *mangiferaeindicae* on yeast dextrose carbonate agar and proved pathogenicity where 3 week old mango seedlings were infiltrated medium using 10µl (10^8 CFU/ml) of each strain suspended in sterilized water. Typical symptoms of mango bacterial leaf spot were observed on all the strains assayed 2 weeks after inoculation.

Najafipour *et al*. (2014) isolated *Xanthomonas campestris* pv. *mangiferaeindicae* from the mango strain using sucrose nutrient agar. They tested for pathogenicity by infiltration method using a sterile needle injected to young leaves of mango seedlings through artificial wounds. Treated plants showed progressive necrosis symptoms within 3 weeks and the absence of these symptoms in control were evaluated as positive bacterial pathogenicity.

Zombre *et al.* (2016) isolated non pigmented *Xanthomonas* like colonies on KC medium. They proved pathogenicity on attached leaves of 6-month old potted mango cv. Maison rouge from the youngest vegetative flush and were

infiltrated (10 inoculation sites per leaf with three replicate leaves on three different plants per bacterial strain) with suspensions of togo strains. Inoculated plants produced typical bacterial canker symptoms a week after incubation in a growth chamber at 30 ± 1˚C day and 26 ± 1˚C (12-h day/night cycle) at 80 ± 5% relative humidity and no lesions were recorded from negative control.

Fatima *et al*. (2019) carried out pathogenicity test for confirmation of *X. axonopodis* pv. *citri* on grape fruit by spray inoculum method. The inoculum was sprayed equally on all plants and again covered the plants with polythene sheets for 2 hrs. Indicator plants were kept under observation for 15 days of post inoculation. After 15 days after inoculation results showed that the isolate was virulent in nature and showed pathogenicity through lesion formation and canker symptoms on leaves of citrus plants. For further clarification when bacterium was re-isolated from diseased tissue and carried the procedure of isolation and purification it showed same colony character of original bacteria and was pathogenic bacteria.

Bhure *et al.* (2019) studied pathogenic variability in pot culture (seedling inoculation) technique (*in vivo*) and detached leaf technique (*in vitro*) among ten isolates of *X. axonopodis* pv. *citri* isolated from different regions of Vidharbha. Results showed that all isolates exhibited symptoms between ranges of 10 (Xac10) to 15 DAI *in vitro* and 15(Xac10) to 25 DAI *in vivo* respectively and conformed that isolate Xac-10 showed highly pathogenic to initiate water soaked lesion and fully developed symptoms after 10 days under *in vitro* and 15 days under *in vivo* condition. While Isolate Xac-10 (Akola) gave 3 mm water soaked lesions surrounded by yellow halo zone. No symptoms were observed in control plate inoculated with sterilized water by pin prick method. While Xac-2, Xac-3, Xac-5, Xac-6 and Xac-7 were not found any canker lesions however, Xac-1, Xac-4, Xac-8 and Xac-9 were found moderate lesions of canker under *in vitro* condition. Whereas, Xac-2, Xac-3, Xac-5, Xac-6 and Xac-7 were found weak canker lesions and Xac-1, Xac-4, Xac-8, and Xac-9 were found moderate canker lesions on leaves under *in vivo* condition.

2.4.2. Morpho-cultural characters

Manicom and Wallis (1984) described that the bacterial cells were Gram-negative, aerobic, non sporulating rods (0.4-0.5 x 1.0-1.5 µm) and motile by means of a single polar flagellum. Colonies on nutrient agar or King's medium B are round, shallow, convex having entire margins. The colony colour was initially smoke grey but soon changes to creamy-white. On potato dextrose agar, the colonies were circular, smooth, glistering, pulvinate having entire margins and measures 1.0-1.5 cm in diameter after 7 days of incubation. The bacterium was strictly oxidative. The optimum temperature for growth was 27^0C but the growth occured up to 37^0C. The thermal death point was around 55^0C.

McGuire (1988) identified six isolates as *Xanthomonas campestris* pv. *vesicatoria* on the basis of morphological tests. All the isolates were, rod shaped, motile, aerobic, all six isolates grew on tween medium.

Manjula *et al.* (2002) reported that seven isolates of the bacterium *X. axonopodis* pv. *punicae,* causing pomegranate bacterial blight were small rods, appeared singly, Gram negative, non-capsulated and non spore forming with monotrichous flagellation.

Graham *et al*. (2003) reported *Xanthomonas axonopodis* pv. *citri* was Gram negative, slender, rod shaped, aerobic, motile by a single polar flagellum, produced slow growing and non-mucoid colonies.

Ah-You *et al*. (2007) described *X. campestris* pv.*mangiferaeindicae* was a gram negative bacterium and produced non pigmented colonies on agar media.

Sain and Gour (2008) studied different morpho-cultural characters *viz.,* cell morphology, pigmentation, elevation, margins, density of bacterium isolated from leaf blight of *Parthenium hysterophorus* and identified as *X. axonopodis* pv. *parthenii.* Results revealed that, the colonies were straw yellow coloured, smooth, glistering with entire margin and convex elevation on NA medium while, bacterium was single polar flagellate, capsulated, non-spore former and acid fast negative.

Khalid and Sinha (2008) reported that, all the isolates of *Xanthomonas oryzae* pv. *oryzae* grew fast on Wakimoto's medium as compared to other media. Maximum (18.0 mm) growth was observed in Xoo 9 isolate; whereas, minimum (11.0 mm) growth was observed in Xoo 19 isolate. Isolates failed to show variability in Gram reaction, cell shape and colony type. But pigmentation of colonies varied from whitish yellow (Xoo 4, Xoo 9, Xoo 10) to straw yellow (Xoo 11, Xoo 12 and Xoo 18).

Myung *et al.* (2009) isolated two isolates (BC2642 and BC2923) of the bacterium *X. perforans* causing bacterial leaf spot of tomato. The isolates were gram-negative, aerobic rods with a single flagellum. On peptone sucrose agar, colonies were yellow and raised with smooth margins. Starch and pectale hydrolysis tests were positive.

Thind (2012) described the bacterium *X. citri* pv. *mangiferaeindicae* possessed all the characteristics of genus *Xanthomonas* except the production of yellow pigment. It produces non pigmented (creamy-white) colonies on agar medium. However, a few yellow-pigmented strains have been isolated from mango in Brazil, Florida, South Africa and Reunion Island. A typical strains, including yellow-pigmented strains collected from various countries and non-pigmented strains isolated from mango in Brazil and from ambraella (*Spondias cytherea* Sonn.) in the French West Indies were phenotypically and genetically distinct from *X. citri* pv. *mangiferaeindicae*.

Thirumalesh (2012) reported that isolates of *Xanthomonas campestris* pv. *mangiferaeindicae* were gram-negative, non-spore forming, motile and small rods singly or in pairs and produced white to yellow, mucoid, circular, raised 1 to 2 mm colonies on YNA and NA medium.

Singh and Thind (2014) studied morpho-cultural characters of isolate *X. axonopodis* pv. c*itri*. Results revealed that, the isolated bacterium was round with creamy to yellowish colonies, gram-ve, rod shaped with high motility.

Iqbal *et al.* (2016) studied morphological characteristics of *Xanthomonas axonopodis* pv *citri* causing citrus canker. Sample collected studied

with respect to characters *viz.,* colour on NA, color on PDA, growth time, shape, elevation, surface, and margin of media growth. They reported that all the isolates were appeared as stack yellow in colour on NA medium where pale yellow on PDA medium. While all the isolates grew with circular shape, convex elevation, mucoid and glistering surface and having entire margin.

Abhang *et al.* (2018) compared total seven isolates of *X. axonopodis* pv. *citri.* on the basis of their colony colour, shape and Grams staining reaction. The results revealed that, bacterial cells appeared short rod and Gram negative. Isolates Xac1, Xac2, Xac3 produced yellow colonies however, Xac 4, Xac 5, Xac 6, Xac 7 showed pale yellow colour colonies on NA medium.

Al- Dulaimi *et al.* (2018) studied 35 isolates of *X. axonopodis* pv. *citri.* on the basis of morphological and cultural characteristics *viz.,* cell shape, gram's reaction and pigmentation on NA medium. Studies revealed that, colonies of all isolates of *X. axonopodis* pv. *citri* after 48 hr of incubation at 28-30^{0}C appeared as circular, mucoid, convex, yellow to orange color while cell shape of all isolates were rod shaped.

Kharde *et al.* (2018) studied isolates of *X. axonopodis* pv. *citri* collected from different location of Maharashtra state. Isolates were studied with respect to their colony colour, shape and gram staining reaction. Bacterial cells appeared rod shaped and gram negative.

Bandi (2019) studied morphological variability among the isolates of *Xanthomonas campestris* pv. *mangiferaeindicae* collected from Marathwada region. All the isolates were rod shaped with rounded ends. Cells single and paired sometimes, gram negative, non spore forming. Also studied cultural variability on nutrient agar medium. The results revealed that the isolates differed with respect to colony characters such as size, shape, colour and appearance. Xcm1, Xcm2 and Xcm5 developed small to medium bacterial colonies. The isolates Xcm3, Xcm4, Xcm7 and Xcm8 developed medium to large colonies. The shape of colonies of the isolates Xcm1, Xcm3, Xcm5, Xcm6, Xcm7 and Xcm8 were having circular to irregular shape. Xcm2 and Xcm4 were having circular in shape. Isolates Xcm1, Xcm2, Xcm5 and

Xcm7 were creamish white and isolates Xcm3, Xcm4, Xcm6 and Xcm8 formed creamish colonies.

2.4.3. Biochemical characters

McGuire (1988) identified six isolates as *Xanthomonas campestris* pv. *vesicatoria* on the basis of biochemical tests. All the isolates were gram-negative oxidase negative, catalase positive and amylolytic positive. All six isolates grew on tween medium.

Thimmegowda *et al.* (2008) studied total 18 isolates of *Xanthomonas oryzae* pv. *oryzae* on the basis of biochemical tests *viz.,* gram negative, gelatin liquefaction, hydrogen sulphide production, starch hydrolysis, oxidase reaction, catalase test and gelatin hydrolyze test. Study revealed that, the isolates Xoo1, Xoo2, Xoo3, Xoo4 and Xoo5 showed positive reaction for gelatin liquefaction and hydrogen sulphide production but the isolates Xoo4 and Xoo5 were negative for starch hydrolysis. All the 18 isolates of bacterial blight of rice in Iran were gram negative, positive for oxidase reaction, catalase and capable of hydrolyzing gelatin.

AL-Saleh (2010) isolated five bacterial isolates from the infected tomato seedlings. The pathogen isolated was confirmed biochemically and physiologically. All isolates were oxidase positive and levan negative, arginine-dihydrolase positive and not macerate potato discs.

Kale *et al.* (2012) studied total 11 biochemical test on isolate of *X. axonopodis* pv. *punicae* causing oily spot of pomegranate. They reported that the test bacterium was positive for potassium hydroxide (KOH), weakly positive to oxidase test and negative to gram iodine test. While it was unable to reduce nitrate or to produce hydrogen sulphide and indole. They also reported that inability of bacterium to utilize Asparine as a carbon and nitrogen source and inhibition on 0.1% tryphenyle tetrazolium chloride (TTZ).

Thind (2012) studied the tests like esculin hydrolysis, caseinase, catalase, cellulose, gelatinase, hydrogen sulphide, levan and lipase production and starch hydrolysis which was positive but tests like acetoin production, arginine dihydrolase activity, indole and ammonia production, 2-ketogluconate production,

urease production, growth on asparagines assource of C and N, nitrate production, cytochrome oxidase, poly-β-hydroxybutyrate synthesis was negative for *Xanthomonas campestris* pv. *mangiferaeindicae.* The bacterium produced acid from glucose, sucrose, xylose, galactose, mannose, D-ribose, trehalose, cellobiose, melibiose, esculin, dextrin, glyseron and sorbitol when growth occurs utilizing these compounds.

Yasuhara *et al*. (2012) reported that strains of *Xanthomonas campestris* pv. *mangiferaeindicae* were non pigmented, gram negative, oxidative, positive for both starch and esculin hydrolysis, negative for nitrate reduction.

Sain and Gour (2013) isolated bacterium from soybean and identify as *X. axonopodis* pv. *glycines* and studied different biochemical characters *viz.,* Gram's staining, KOH solubility, nitrate reduction, gelatin liquefaction, arginine dihydrolase, catalase and milk proteolysis. Results revealed that, bacterium was KOH soluble and negative for indole production, arginine dihydrolase and nitrate reduction while, the bacteria liquefied starch, gelatin and casein.

Rafi *et al.* (2013) characterized 120 isolates of *X. oryzae* pv. *oryzae* causing bacterial blight of rice on the basis of different biochemical tests *viz.*, Gram's staining, 3% KOH test, starch hydrolysis test, tween 80 hydrolysis test, acid production from carbohydrates, egg yolk reaction, tetrazolium salt tolerance test, anaerobic growth test and oxidase test. Results revealed that, these isolates were KOH positive and radialy hydrolyze tween -80 and starch, form acid from carbohydrates, their egg yolk reaction being negative. Further, these show no tolerance to 1% terazolium negative, anaerobic test negative and oxidase test negative.

Najafipour *et al.* (2014) identified *Xanthomonas citri* pv. *mangiferaeindicae* based on phenotypic and biochemical tests where small white and yellow , slightly mucoid or non-mucoid, gram negative and obligate aerobe colonies were observed on sucrose nutrient agar medium. All strains were oxidase negative, but catalase, levan production, gelatin, starch and esculin hydrolysis, tween 80, litmus milk, hypersensitive reaction on geranium, Hydrogen sulphide production tests were positive.

Arshiya *et al.* (2014) studied biochemical characters among 20 isolates of *X. axonopodis* pv. *citri* causing citrus canker with 18 different tests *viz.,* starch hydrolysis, gelatin liquification, aesculin hydrolysis, tween 80 lipolysis, milk hydrolysis, oxidase test, H_2S production, urease production, arabinose production, fructose utilization, sucrose utilization and trihalose utilization. They reported that all the isolates were negative to oxidase test; while others are positive.

Al – saleh *et al.* (2014) studied total 76 isolates of *X. citri* subsp. *citri* strains subjected to different biochemical test *viz.,* Gram reaction, Oxidative / Fermentation test, Arginine dihydrolase, Fluorescent pigmentation, NaCl tolerance, Nitrate reductase, hydrolysis of casein, gelatin, starch, tween 20 and 80. They reported positive reaction of *X. citri* subsp. *citri* to hydrolysis of casein, gelatine, starch, tween 20 and 80, while negative to gram reaction, Arginine dihydrolases fluorescent pigmentation.

Mubeen *et al.* (2015) studied biochemical characterization of citrus canker infected leaf samples collected *viz.,* Gram reaction, starch hydrolysis, tween 80 hydrolysis, kovacs' oxidase, gelatin liquefaction, fluorescent pigmentation and KOH test. They reported that Gram reactions were found negative; while others all found positive.

Katkar *et al.* (2016) studied pathogenic variability among 15 isolates of *X. axonopodis* pv. *citri,* causing citrus canker in acid lime collected from different agro climatic regions of India. They reported all the test isolates as rod shaped, Gram negative, strongly positive to catalase, KOH, H_2S, acid and gas and gelatin and starch hydrolysis; while the test isolates were negative only to iodole production.

Silva *et al*. (2017) reported that *X. campestris* pv**.** *mangiferaeindicae* strain IBSBF 2103 produced xanthum gum on yeast malt broth medium and the factors that affect the xanthum yield were extensively studied.

Abhang *et al.* (2018) compared total seven isolates of *X. axonopodis* pv. *citri.* on the basis of their biochemical tests *viz.,* starch hydrolysis, Gelatin liquefaction, Indol Production, KOH test, Gram's reaction, Acid and gas production and catalase test. Results revealed that, all seven isolates showed Grams negative

reaction, strongly positive to idole production and catalase test, moderately positive to acid gas production; whereas, three isolates *viz.,* Xac1, Xac2 and Xac3 exhibited strongly positive reaction to starch hydrolysis while Xac 4, Xac 5, Xac 6 and Xac 7 were weakly positive.

Al- Dulaimi *et al.* (2018) studied 35 isolates of *X. axonopodis* pv. *citri.* on the basis of biochemical tests *viz.,* starch hydrolysis, gelatine liquefaction, KOH test, Indol production, oxidaes test, acid and gas production, catalase reaction, fluorescent pigmentation test and salt tolerant test 3% NaCl. Studies revealed that, all the isolates were positive to starch hydrolysis, gelatine liquefaction, KOH test, Indol production, acid and gas production, catalase reaction while, negative to oxidaes test.

Kharde *et al.* (2018) studied isolates of *X. axonopodis* pv. *citri* collected from different location of Maharashtra state. All isolates were positive to starch hydrolysis. In KOH test all isolates formed thread like slime. The isolates were positive for H_2O_2 test and all isolates were observed to start liquefaction of gelatin after seven days. Bacteria utilized dextrose as carbon source and were observed as a positive for production of acid and gas.

Bhure *et al.* (2019) studied biochemical variability among ten isolates of *X. axonopodis* pv. *citri* isolates from different regions of Vidharbha. A several biochemical tests *viz*., Gram staining, catalase test, KOH test, starch hydrolysis, gelatin liquefaction, H_2S production, indole production, acid and gas production etc. were carried out for biochemical confirmation of *X. axonopodis* pv. *citri*. Results revealed that, all isolates were found positive for starch hydrolysis, KOH test, catalase test, H_2S production, gelatin liquefaction, indole production, acid and gas production tests to performed for characterized the *X. axonopodis* pv. *citri* bacteria. The result of all bio-chemical test confirmed that, *X. axonopodis* pv. *citri* was gram negative bacterium.

Bandi (2019) studied biochemical characteristics of the eight isolates *X. campestris* pv. *mangiferaeindicae*. The results revealed that bacterium liquefied the gelatin, hydrolyzed the starch and casein, positive for KOH and catalase tests and produced xanthum gum.

2.4.4. Molecular variability

Gagnevin *et al.* (1997) assessed the genetic diversity among 13 strains of the bacterium isolated from mango, ambraella, and pepper tree in 14 countries and found that 11 strains did not belong to *X. campestris* pv. *mangiferaeindicae*. They further revealed that the remaining 127 strains formed four groups, of which the group with the greatest diversity consisted of strains frpm Southeast Asia where mango obtained. Another group contained only stains isolated from pepper trees in Reunion, indicating that the papper tree may not be a collateral host for the bacterial leaf spot pathogen.

Jeong *et al*. (1997) studied genetic diversity among 44 strains of *X. campestris* pv. *vesicatoria* from diverse geographic origin by RAPD of genomic DNA. Cluster analysis of genetic similarity among the strains generated the dendrogram that clearly separated all strains from each other. A total of 39 Korean strains were classified in to 11 subgroups. Masan strain Ms 93-1 clustered distinctly from the other Korean strains. RAPD polymorphism suggested that the occurrence of genetic differentiation of *X. c.* pv. *vesicatoria* and the existence of genetically distinctive subgroups among the population in Korea.

Gupta *et al.* (2001) studied among sixteen isolates of *X. oryzae* pv. *oryzae* representing different geographical location in India along with 2 isolates from the Philippines using polymorphic RAPDs. The primers OPA - 03, OPA - 04, OPA - 10, OPA - 11, OPK - 7, OPK - 12 and OPK - 17 generated simple, specific and reproducible fingerprint patterns, indicating usefulness of RAPD markers in differentiating *X. oryzae*. pv. *oryzae* isolates. They reported that based on the RAPD-PCR (seven primers) and IS1112 - PCR (two primers) data, at a similarity of 0.57, sixteen out of 18 isolates were closely grouped with them. The data using RAPD-PCR and IS1112 based PCR approaches revealed their potential in rapid identification of isolates, in assessment of genetic variation in the Indian pathogen population and in generating unique DNA fragments specific to 8 isolates of *X. oryzae*. pv. Oryzae.

Loiselene (2005) studied repetitive DNA based- PCR (rep-PCR) profiles were generated from purified bacterial DNA of 40 field strains of *X. campestris* pv. *viticola*, collected between 1998 and 2001 in the states of Pernambuco,

Bahia and Piauí. Combined analysis of the PCR patterns obtained with primers REP, ERIC and BOX, showed a high degree of similarity among Brazilian strains and the Indian type strain NCPPB 2475. Similar genomic patterns with several diagnostic bands, present in all strains, could be detected. Fingerprints were distinct from those of strains representing other pathovars and from a yellow non-pathogenic isolate from grape leaves. The polymorphism observed among the Brazilian strains allowed their separation into five subgroups, although with no correlation with cultivar of origin, geographic location or year collected.

Madani *et al.* (2010) studied genetic variability among 37 isolates of *X. citri* subsp. *malvacearum* causing cotton blight by rep-PCR. genomic finger printing. They reported that PCR amplification of the isolates with ERIC primers produced 21 reproducible bands ranging from 250 to 2500 bp. while 5 genotypes were identified that separated in two main clusters. Whereas *X. citri* subsp. *malvacearum* (reference isolate ICMP 217) showed 90 per cent similarity to four isolates.

Kale *et al.* (2012) studied molecular characteristics of an isolates of *X. axonopodis* pv. *punicae* causing oily spot of pomegranate. Sample collected from Nasik district by plasmid profiling. They used restriction analysis using two restriction enzymes (MBI fermentas) *viz.,* Hind III and Sac I separately. They revealed that restriction digestion of those plasmids with two restriction enzymes, *viz.,* Hind III and Sac I with its size 93.64 kb and 96.18 kb respectively; conforming typical of *Xanthomonas* spp.

Rezaei *et al.* (2012) studied genetic diversity among 25 isolates / strains of *X. citri* subsp. *citri* collected from different provinces of Iran by using two marker systems *viz.,* repetitive polymerase chain reaction (rep-PCR) and random amplified polymorphic DNA (RAPD). They reported that two primers, ERIC 1R and 232, with the highest marker index, resulted in the most genetic variability among strains. While cluster analysis by band pattern showed that strains from sistan-va-βaluchestan province were a different group.

Arshiya *et al.* (2014) studied genetic diversity among 20 isolates of *X. axonopodis* pv. *citri* causing citrus canker by rep. PCR (repetitive DNA polymerase chain reaction based finger printing) using repetitive extragenic

palindromic (rep) and enterobacterial repetitive intergenic consensus (ERIC) and BOX primers. They generated total 13 bands out of this eight bands common in all strains while remaining bands were different in the strains. There was no unique band were observed in the intensity of bands.

Gadhe *et al*. (2016) studied molecular variability among five isolates of *X. axonopodis* pv. *punicae* by using 10 RAPD primers. They reported that 07 primers produced total 26 scorable bands with an average of 3.7 bands per primer. Out of 26 bands, 21 bands were polymorphic, with 80.76% polymorphism. The cluster analysis based on similarity coefficient, the five isolates were categorized into two major and two sub clusters, when indicated high level of genomic variability among the isolates.

Katkar *et al.* (2016) studied pathogenic variability among 15 isolates of *X. axonopodis* pv. *citri,* causing citrus canker in acid lime collected from different agro climatic regions of India by DNA(RAPD) analysis . The reported that out of 27 primers scored 19 showed amplification and produced scorable bands of high degree of polymorphism. Out of 220 amplifications , 218 wre polymorphic with 99.2% level of polymorphism. The banding pattern profile indicated high degree of molecular variability among 15 test isolates. The genetic similarity co-efficient of 15 test isolates was ranged from 0.27 to 0.68, which showed high genetic diversity.

Chavan *et al.* (2017) studied molecular variability among 36 strains of *X. axonopodis* pv. *punicae*, causing oily spot of pomegranate from the diseased fruits of three different varieties of pomegranate originating from three different provinces of Maharashtra, India. They reported that all the strains characterized phenotypically and genotypically were diverse. While the genetic diversity among the 36 *X. axonopodis* pv. *punicae* isolates were assessed using RAPD based techniques. A cluster dendrogram based on the random amplified polymorphic DNA (RAPD) showed that genetic diversity existed among the isolates of *X. axonopodis* pv. *punicae*. The genomic variation was found to be in the range of 0.55% to 0.95% among the isolates.

Kharde *et al.* (2018) studied molecular variability of *X. axonopodis* pv. *citri* collected from different location of Maharashtra state by using 14 RAPD and 8

ISSR primers out of which 7 RAPD markers produced 47 scorable bands and 2 ISSR primers produced 13 scorable bands. The similarity coefficient value ranged from 0.13 to 0.40 for RAPD and from 0.00 to 1.00 for ISSR primers across five isolates indicating high degree of genetic variation.

2.4.5. Host range

Ravikumar and Khan (2000) studied the host range to assess the infection of *X. campestris* pv. *vesicatoria*. The results revealed that the three isolates of *X. campestris* pv. *vesicatoria* from tomato did not cause infection in other crop. Plants and weeds tested *viz.,* bean, bell papper, brinjal, chilli, potato cowpea, tobacco, *Datura stramonium* and *Solanum* sp. except its host *i.e* tomato. However, infection of *X. campestris* pv. *vesicatoria* on tomato were seen 20 days after inoculation.

Ah-you *et al.* (2007) on the basis of pathogenic variation and host range, divided and reclassified *X. campestris* pv. *mangiferaeindicae* into three pathovars, namely, *X. axonopodis* pv. *mangiferaeindicae* pathogenic on mango and cashew, *X. axonopodis* pv. *anacardii* pathogenic on cashew and *X. axonopodis* pv. s*pondiae* pathogenic on ambarella and mombin.

Bhat *et al.* (2010) studied host range under conditions of artificial inoculation. Results revealed that the bacterium infected almost all the crucifer crops cultivated in Kashmir, besides some cruciferous weeds but failed to infect any of the non-cruciferous plant species tested.

Thind (2012) isolated *X. citri* pv. *mangiferaeindicae* from cashew (*Anacardium occidentale* L.) in India. Non- pigmented strains were also isolated from ambarella in the French West Indies and from Brazillian pepper (*Schinus terebinthifolius*) in Reunion. The ambraella strains were not only taxonomically distinct from mango isolates but are also much less aggressive on mango.

2.5. Epidemiological studies

2.5.1. Role of Weather Factors

Effect of seasonal variation in temperature and humidity on bacterial blight 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 *X. malvacearum* (E. F. Smith) opined that, the disease occurred during last week of August or first week of September on two months old crop, when widespread rainfall (300 to 350 mm) occurred at a mean temperature of 22.8°C to 31.5°C and relative humidity between 58 to 78 per cent prevailed. Thereafter, the infection rate declined from first week of November onwards with the decrease in mean temperature (19.1-30.3°C), relative humidity (40-78%) and rainfall.

Aubert (1981) stated that high crop losses (80%) due bacterial leaf spot of mango can occur, especially after a cyclonic depression causing high and heavy rains.

Manicom (1986) stated that bacterial black spot of mango caused by *X. campestris* pv. *mangiferaeindicae* was found to be essentially a wound pathogen of leaves under glasshouse conditions, with maximum infection occurring at a temperature regime of 22/26 ^{0}C (night/day). The organism was a phylloplane resident year round in the field and inoculum levels in the tree canopy affect ultimate fruit infection. The major weather factor affecting fruit infection was rainfall which showed a significant correlation (r = 0–77) with levels of infection after allowing for an approximate 2 week latent period.

Chand *et al.* (1991) studied the effect of different dates of pruning on the intensity of bacterial canker of grapevine caused by *X. 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 it to the favourable weather conditions during early September, for disease development. The frequent rains during this period helped pathogenesis and further spread of bacteria through splash action. The minimum (25°C) and maximum (30°C) temperature prevailed during first fortnight of September were also congenial for canker development.

Chandra *et al.* (1994) studied that 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 *X. 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. He also recorded the minimum temperature range between 17.0°C-19.4°C, maximum temperature 40.4°C-48.0°C and relative humidity between 25-100 per cent during May in all these consecutive years from 1988-90.

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

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°C-19.4°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, 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°C-42.9°C and minimum temperature between 20.8°C – 24.2°C). The disease severity coincided with the fruit development and fruit maturity stage, where 90 per cent of developing fruits got infected resulting in huge loss in yield and quality.

Katwal (2015) studied correlation between bacterial blight of pomegranate and weather factors. Results revealed that, maximum temperature had

significant negative correlation with the disease development in 2012 and 2013. Role of minimum temperature was significant and positive with the disease development. Relative humidity and rainfall had positive significance relationship with the disease in both the years. Step-wise regression models showed strong and positive relationship between combined effects of weather factors for disease development. The apparent infection rate of the disease was slow initially which gradually increased when temperature and humidity became favourable in both the years. The area under disease progress curve (AUDPC) was more on leaves as compared to fruits.

2.6. Disease management strategies

2.6.1. Bioefficacy of antibiotics and chemicals

Pal *et al.* (1981) evaluated the sensitivity of five phytopathogenic bacteria against antibiotics and fungicides. The reported that paushamycin alone and in combination with copper oxychloride resulted with maximum inhibition of growth of *X. citri*. Kerosene oil emulsion @ 4% was found to be superior to plantomycin and paushamycin.

Sharma *et al.* (1981) obtained the highest inhibition effect on the growth of *X. vesicatoria* by the combined treatment of streptocycline and copper sulphate.

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

Hernandez (1991) reported that sodium hypochlorite, chlorhydric acid, sodium hypochlorite and at 2% and 3% were used to control of *X. campestris* pv. *vesicatoria* in artificially inoculated seed of the tomato variety L-72-1. Soaking seed in sodium hypochlorite or in the combination of sodium hypochlorite and chlorhydric acid for 20 min. gave the best disease control.

Chirame and Shinde (1993) evaluated *in vitro* efficacy of some antibiotics against *X. compestris* pv. *citri* and reported highest zone of inhibition with streptocycline @ 500 ppm and it was least significantly with aureofungin @ 10 ppm.

Sheikh *et al. (*1995) reported that among 10 toxicants tested against *X. campestris* pv. *mangiferaeindicae*, agromycin-(streptomycin) at 1% was the most inhibitory.

Boshoff (2000) described control measures against bacterial black spot of mango. Agry-gent a commercial plant formulated antibiotic consisting of 100 g gentamycin / kg and 300 g oxytetracycline/kg, in combination with copper oxychloride spray applications was evaluate as alternative control strategy. The results indicated significant disease control with Agry-gent used at 75,100 and 200 g/100 litre.

Graham *et al.* (2004) conducted field trials in Brazil, demonstrated that even reduced rates of copper formulations (copper hydroxide, copper oxychloride) were consistently effective for control of citrus canker (*X. axonopodis* pv. *citri.*) on moderately susceptible orange varieties. They also reported that fungicide famoxate with reduced rates of copper hydroxide did not show greater control activity than the lower rates of copper hydroxide alone.

Hasan (2005) studied *in vitro* activities of eleven chemicals (blasticidin, celdion, tricyclazole, streptomycin, sumithione, Saturn, mipcine, stem F-34, hinosan, kasumin and phytomycin) against bacterial blight of paddy caused by *X. campestris* pv. *oryzae*. Six chemicals (phytomycin, streptomycin, blasticidin, kasumin, tricyclazole, and sumithione) arrested the growth of bacteria *in vitro* and produced good inhibition zone.

Shahid *et al. (*2005) tested *in vitro* the toxicants *viz.,* streptomycin sulphate, dithane M-45, agrimycin – 100, vitavax, benlate and cobox (each @ 0.01, 0.1 and 1% concentration against *X. campestris* pv. *citri*. They reported that agrimycin –100, streptomycin sulphate, vitavax, and dithane M-45 as most effective with significantly maximum inhibition of the bacterium. Streptomycin sulphate, agrimycin – 100, vitavax, dithane m–45 and benlate at 0.2% concentration were sprayed on the field grown citrus plants and then inoculated with *X. campestris* pv. *citri* for control of citrus canker disease. Streptomycin sulphate, vitavax, dithane m-45 and agrimycin-100 in the order proved effective also in reducing the disease intensity as compared to inoculated control.

Giri *et al*. (2008) evaluated copper oxychloride (0.2% and 0.3%) + Streptocycline (@ 100 ppm and 200 ppm) aginst *X. axonopodis* pv. *citri*. Among that the minimum disease intensity (12.29%) with maximum disease control (37.63%) was recorded with three spray of copper oxychloride + streptocycline (0.3% + 100ppm).

Dhakal *et al.* (2009) tested *in vivo* the different chemicals *viz.,* copper oxychloride (2.5%), copper oxychloride + kasugamycin (1000X), bordeaux mixture 1% and 2% in citrus orchard at Dhulikhet and the decrease in disease severity after spraying of the chemicals was recorded with reference to the plants that were not sprayed with the chemicals.

Kumar *et al.* (2009) evaluated *in-vitro* efficacy of different chemicals against *X. oryzae* pv. *oryzae* causing bacterial blight of rice, among which the chemicals copper oxychloride (0.25%) + streptomycin sulphate (200 ppm) showed maximum inhibition zone.

Samavi *et al*. (2009) tested the efficiency of two novel compounds *i.e.* Thyme essential oil (TEO) produced from Zaatar (*Zataria multiflora*) and Nanosilver (NS), as well as some commonly used chemicals against citrus bacterial canker using detached-leaf assays in *in vitro* and whole seedlings in the greenhouse. Mexican lime seedlings were used for detached leaf assays. Treatments included 0.3% copper oxychloride (COC), 1.5% bordeaux mixture (BO), 0.3% COC+0.04% mancozeb (MZ), 1.5% BO+0.04% MZ, TEO at 10-2 dilution, three concentrations of NS (100, 150 and 200 ppm) and 100 ppm streptomycin (S). The BO+MZ, COC+MZ and TEO treatments were found to be most effective based on mean number of lesions and type of symptoms on detached leaves.

Dhutraj and Suryawanshi (2010) tested antibiotics in *in vitro* conditions *viz.,* streptocycline, aureofungin, plantomycin and blastosan (each @ 100, 150, 200 and 250ppm) against *X. axonopodis* pv.*vesicatoria* by poisoned food technique. They reported streptocycline as more effective with growth inhibition of 48.00%, 55.33%, 52.66% and 61.00% @ 100, 150, 200 and 250ppm, respectively. This was followed by aureofungin with 45.00%, 50.66%, 52.66% and 54.66% plantomycin 40.66%, 43.66%, 46.00% and 45.31% and bactosan with 34.33%,

36.00%, 39.00% and 40.33% growth inhibition @ 100, 150, 200 and 200 ppm, respectively.

Jambenal *et al*. (2011) tested *in-vitro* efficacy of different chemicals against *X. campestris* pv. *viticola* causing bacterial leaf spot of grape where the streptocycline 500 ppm plus copper oxychloride 2000 ppm produced maximum inhibition zone (24.97 mm) followed by streptocycline 500 ppm (22.40 mm).

Thirumalesh *et al.* (2011) presented the results obtained from *in vitro* studies showed that the antibiotics ciprofloxacin,tetracyclin, and kanamycin were the strongest effect against the four tested strains of *X. campestris* pv. *mangiferaeindicae* whereas chloramphenicol, copper sulphate, copper sulphate, copper oxychloride and commercial bacterinashak also exerted in vitro antibacterial activity.

Thirumalesh *et al*. (2012) carried out experiments *in vitro*, six commercial formulates (copper sulphate, copper oxychloride, streptocycline, mancozeb, bactrinashak and bavistin) were evaluated against four strains of *X. campestris* pv. *mangiferaeindicae*.They reported that all compounds inhibited bacterial growth afterhours incubation, except bavistin which was not active at 1000µl/ml concentration and the mixed compounds were evaluated in pairs copper oxychloride + copper sulphate, streptocycline + bactrinashak, mancozeb + copper oxychloride, mancozeb + bavistin and bavistin + bactrinashak ; for those combinations sub inhibitory concentrations of each component drastically reduced colony forming units(cfu)counts(>99.9%) from initial inoculum.

Raju *et al.* (2012) carrried out investigation to screen the different bactericides aginst *X. axonopodis pv. punicae*. to inhibit zone of 3.3cm exibited superior efficacy followed by streptocycline (2.80cm) and copper oxychloride (2.65cm).

Raghuwanshi *et al.* (2013) studied four different test isolates of *X. axonopodis* pv. *punicae* in *in vitro* conditions collected from Solapur, Sangali, Ahmednagar and Nasik districts to observe the complete control in all the test isolates with different chemicals at different concentrations *viz.,* bordeaux mixture (1%); captan (0.25%) + copperoxy chloride (0.3%), captan (0.25%) + copper hydroxide

(0.3%), Bromopol (500 ppm) + copperoxy chloride (0.3%), streptocycline (250 ppm) + copper hydroxide (0.3%), streptocycline (500 ppm) + copper hydroxide (0.3%). Result showed that streptocycline 250 ppm, has most variable response with per cent growth inhibition of 58.60 % and 99.21 % to the test isolates collected from the Sangamner and Pandhapur-Solapur area.

Sajid *et al.* (2013) tested three different chemicals *in vitro* using inhibition zone technique against *X. axonopodis* pv. *malvacearum* causing bacterial blight of cotton. Among them plant protector was found most effective at 600 ppm after 72 hours of treatment compared to others.

Yenjerappa *et al.* (2014) carried out *in vitro* evaluation of bactericides and antibacterial chemicals on *X. axonopodis* pv. *punicae*. They reported the results that was significant superior efficacy of bactinash-200 with an inhibition zone of 15.07mm followed by bronip(14.67) and plantomycin(13.77) with par efficacy between each other.The other chemicals *viz.,* K-cycline, copper oxychloride, Bordeaux mixture, bactrinashak, bleaching powder and streptocycline, exhibited par moderate efficacy each other.

Katwal (2015) reported that among different chemicals and antibiotics tested *in vitro* against *X. axonopodis* pv. *punicae* causing bacterial blight of pomegranate, streptocycline (100 ppm) produced maximum inhibition zone 12.90 mm in diameter followed by Bordeaux mixture (100 ppm) 12.36 mm in diameter.

Thind and Singh (2015) evaluated six agrochemicals *viz.,* Bactrinashak, Blitox, Bordeaux mixture, Copper sulphate, Kocide and Streptocycline @ 5, 10, 20, 50, 100, 200, 500 and 1000 ppm concentrations *in vitro* and *in vivo* against *X. axonopodis* pv. *citri* causing citrus canker disease. Results revealed that, streptocycline was found to be the most effective at lower concentration (5 ppm) followed by Blitox at 50 ppm. A significantly minimum canker intensity (13.67%) and highest disease control (68.45%) was achieved with streptocycline at 100 ppm in Kinnow mandarin.

Negi and Kumar (2015) evaluated total seven antibiotics against *X. axonopodis* pv. *citri*. Results showed that, all the seven antibiotics tested were

significantly effective. Among these, at 2000 ppm concentration, Chloramphenicol and Streptocycline showed maximum zone of inhibition *i.e.* 2.83 cm and 2.75 cm respectively, followed by Tetracyclin (2.73cm) and Streptomycin (2.45 cm), while Penicillin was found to be least effective at 2000 ppm.

Ambadkar *et al.* (2015) studied *in vitro* efficacy of total seven antibiotics *viz.,* Streptomycine, Streptocycline, Rifamycin, Bacterinol, Tetracycline, Chloramphenicol and Cefachlore at each 250 and 500 ppm against *X. axonopodis* pv. *punicae* causing bacterial blight of pomegranate using inhibition zone technique. They reported that highest per cent inhibition of bacterial growth with strptocycline 22.2% and 31.60% with concentration @ 250 ppm and 500 ppm respectively followed by tetracycline 18.26% and 27.53% @ 250 ppm and 500 ppm respectively. However lowest per cent inhibition were recorded with cefachlore *i.e.* 13.08 % and 17.53 % with concentration @ 250 ppm and 500 ppm respectively.

Badiger *et al.* (2016) evaluated six antibiotics, two antibacterial chemicals *in vitro* against *X. axonopodis* pv. *citri* by inhibition zone assay method with concentration @ 0.05 and 0.1 per cent. Results showed that among the different antibiotics and antibacterial chemical evaluated, streptocycline (10.84 mm) and copper oxychloride (7.50 mm) showed maximum inhibition zone followed by K cycline (9.68 mm) at 0.05 per cent. Also, reported that streptocycline and K cycline @ 0.1 per cent concentration were highly effective with an inhibition zone of 18.96 mm and 17.86 mm, respectively followed by copper oxychloride @ 1 per cent concentration (13.66 mm).

Madavi *et al.* (2016) studied the *in vitro* efficacy of total three antibiotics *viz.,* Streptomycin sulphate, Bromopol and Kasugamycin @ 100, 250 and 500ppm each and two chemicals *viz.,* Vitavax and COH @ 0.1, 0.2 and 0.3 per cent concentration each against eight isolates of *X. axonopodis* pv. *citri* (Xac 1 to Xac 8) by Paper disc method. The results revealed that Treatment T3 *i.e.* streptomycin sulphate (500 ppm) was significantly superior over rest of the treatments showing maximum inhibition zone (19.73 mm) for Xac3, followed by Xac6 (16.53 mm); whereas, it was minimum in Xac1 (7.46 mm). The lowest zone of inhibition was found in treatment T5 (5.40 mm) of Bromopol (250 ppm) for Xac1. Streptomycine

sulphate (100 ppm), bromopol (100 ppm) and COH having concentration 0.1, 0.2 and 0.3 % did not show any inhibition zone to the pathogen.

Savitha *et al.* (2016) studied on integrated management of citrus canker through sanitation and pruning followed by different combinations of copper fungicides, bactericides, ISR chemical, botanicals and bioagent. The results of the experiment revealed that, crop sanitation followed by spraying of streptomycin sulphate (500 ppm) + COC (3g/l) was found effective with per cent disease index (PDI) of 30 and it was on par with sanitation followed by Bordeaux mixture (1%) spray followed by bacterinashak (0.5g/l) and *Pseudomonas fluorescens* (5g/l) spray with PDI of 32.17.

Rashid *et al.* (2016) evaluated *in vitro* efficacy of different chemicals (score, bowek, success and ridomel gold) against *X. campestris* pv. *mangiferaeindicae*. They reported that among those chemicals, at 3% concentration, Ridomil-gold was more significant inhibited where 1.35cm bacterial colony growth.

Thakre *et al*. (2017) studied bioefficacy of six chemical to control *X. axonopodis* pv. *citri* causing citrus canker in field trial, over the season on acid lime tree. The chemicals were Bordeaux mixture @ 1%, Fytolon @ 0.3%, streptocycline @ 200 ppm, Bordeaux mixture (1%)+ streptocycline (200 ppm) and fytolon (0.3%) + streptocycline (200 ppm). Results revealed that, Bordeaux mixture+ streptocycline proved more effective as compared to other. With four application of aques solution of Bordeaux mixture (1 %) amended with Streptocycline (200 ppm) the incidence of canker on leaves was minimum (4 %) as compared to check where 39 percent infection was recorded.

Preecha *et al*. (2018) screened *in vitro* four antibiotics *viz.,* tetracycline (0.1 mg/ml), streptomycin (0.1 mg/ml), ampicillin (0.1 mg/ml) and validamycin (0.03 mg/ml) and two fungicides *viz*., copper hydroxide (0.57 mg/ml) and copper oxychloride (0.63 mg/ml) against *X. axonopodis* pv. *citri* causal agent of canker on *Tabtimsiam pummelo* by agar diffusion technique. The result indicated that tetracycline showed highest control with 1.12 cm inhibition zone, followed by streptomycin (1.02 cm) and ampicillin (0.95 cm). It was also higher than broad

spectrum fungicide, copper hydroxide and copper oxychloride of 0.57 cm and 0.46 cm inhibition zone respectively.

Bandi (2019) studied in vitro evaluation of antibiotics, chemicals and their combinations against *X. campestris* pv. *mangiferaeindicae*, indicated that streptocycline (22.40 mm) followed by carbendazim + streptocycline (21.96 mm) were highly effective in recording maximum mean inhibition zone. Individual concentration of the treatments tested *in vitro* revealed that, streptocycline 500 ppm (27.6 mm) followed by streptocycline + copper sulphate 250 + 2000 ppm (25.3 mm) were found superior showing maximum inhibition zone and inhibited growth of the pathogen to the extent of 30.66 and 28.11 per cent respectively, copper oxychloride (21.60 mm), mancozeb (19.73 mm), carbendazim (18.73 mm), mancozeb + copper oxychloride (18.53 mm). The minimum inhibition was made by copper sulphate treatment (14.20 mm).

Jadhav (2019) tested total seven fungicides (each @ recommended field dosages, 50% of recommended and 125% of recommended field dosages) *in vitro* by applying inhibition zone assay technique against *X. axonopodis* pv. *citri*. Average inhibition zone was significantly highest with copper oxychloride 50% WP (12.75 mm), followed by mancozeb 75% WP (11.61 mm), cyamoxanil 8% + mancozeb 64% WP (11.16 mm), chlorothalonil 75% WP (10.61 mm); whereas, it was significantly least with carbendazim 50% WP (9.92 mm).

2.6.2. *In vitro* evaluation of bioagents :

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

Gallardo *et al.* (1989) reported the *in vitro* inhibition of *P. solanacearum* BC-8 strain of *P. fluorescens*. The growth of *P. solanacearum* on agar was inhibited by inoculation with *P. fluorescens* (Str BC-8) or by application of *P. fluorescens* culture extracts. It was found that the extracts contained rod shaped particles with a helical structure 150 mm long and 25 mm in diameter. These were interpreted as bacteriocins and designated as fluocin BC-8. Bacterioxin activity was

found to be associated with plasmids since plasmid DNA was not detected electrophoretically.

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

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

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

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

Manjula *et al.* (2002) tested antagonistic effect of *P. fluorescens, B. subtilis*, *P. aeruginosa* and *Lactobacillus* spp. on the growth of *X. axonopodis* pv. *punicae,* by inhibition zone 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) reported that among 21 rhizobacterial and 52 phylloplane bacterial isolates isolated from mungbean, the isolates MRb-1 (rhizobacteria), Plb-1, Plb-2 and Plb-3 (phylloplane bacteria) exhibited maximum inhibition of *X. axonopodis* pv. *vignaeradiatae,* tested by dual culture technique on sucrose peptone agar medium. Based on the morphological and biochemical characteristics, MRb-1 isolate was identified as *Pseudomonas fluorescens* and Plb isolates were identified as *Bacillus* spp.

Giri *et al.* (2008) evaluated *in vivo* three bioagents *viz., Trichoderma harzianum*, *Pseudomonas fluorescens* and *Aspergillus niger* against *X. axonopodis* pv. *citri* causing citrus canker. Results revealed that, most effective antagonist was *A. niger* with 17.69 per cent disease control followed by, *P. fluorescens* (16.67%). The least effective antagonist among them was *T. harzianum* exhibitated 15.30 per cent disease control.

Khodakaramian *et al.* (2008) studied *in vitro* bioefficacy of total twenty *P. fluorescens* (Pf) and *P. putida* (Pp) isolates against *X. axonopodis* pv. *citri* causing citrus bacterial canker by inhibition zone technique and most effective isolates (five) of *Pseudomonas* found during *in vitro* study further evaluated in green house against citrus bacterial canker in pot culture by spray inoculum technique. Results revealed that the highest average inhibition zone belonged to strain Pf-19 (6.40 cm) followed by and strain Pf-09 (6.16 cm); whereas, selected strains fro green house studies were promising and reduced no of disease spots between 23.8 to 64.0 per cent.

Kumar *et al*. (2009) reported that among four different bioagents tested *in vitro*, *P. flourescens* and *Trichoderma harzianum* restricted maximum growth of *X. oryzae* pv.*oryzae* causing bacterial blight of rice.

Yenjerappa (2009) studied antagonistic activity of different bioagents *in vitro* aginst *X. axonopodis* pv. *punicae*. Results revealed that, *Bacillus subtilis* and *P. fluorescens* were found significantly superior over other antagonists in inhibiting the growth of *X. axonopodis* pv. *punicae*. The fungal biocontrol agents *viz., Trichoderma viride* and *T. harzianum* were totally ineffective.

Singh *et al*. (2010) evaluated two bioagents such as *P. flourescens* PF-1 and *B. subtilis* strain BS-7 *in vitro* against *X. campestris* pv. *campestris* causing black rot disease of cauliflower. Maximum inhibition zone (2.4 cm) was observed when *P. flourescens* PF-1 containing 4.3x and *X. campestris* pv. *campestris* diluted 10,000 fold.

Jambenal *et al.* (2011) tested *in-vitro* efficacy of two bioagents namely *P. flourescens* and *B. subtilis* against *X. campestris* pv. *viticola* causing bacterial leaf spot of grape. Among them, *P. flouresens* recorded highest inhibition of radial growth (8.15 mm) than *B. subtilis* (7.05 mm).

Thirumalesh (2012) evaluated two bioagents *Trichoderma* sp. and *Lactobacillus* sp. *in vitro* against *X. campestris* pv. *mangiferaeindicae* causing mango bacterial leaf spot. Among them, *Trichoderma* sp. Produced maximum inhibition zone of 3.00 mm and *Lactobacillus* sp. showed zero inhibition.

Gamangatti and Patil (2013) reported that among different isolates of bioagents, *P. florescens*(pf4) was found significantly superior inhibiting(15.73mm) the growth of *X. axonopodis* pv.*punicae* but remained on par with bacillus subtilis BS1 and BS2 with 12.1 and 13.66mm respectively. Whereas bioagents BS3 and Pf6 showed lower inhibitory zone of 0.76 and 0.93mm respectively.The isolates of bioagents BS4, Tv-16, Tv-R, Th-10 and Th-r recorded zero inhibition zone.

Das *et al*. (2014) evaluated one bacterial isolate *Bacillus subtilis* on acid lime, agaist citrus canker during 2009-10. Single pray of the bacterial aqueous suspention was crried out on five batches (six numbers of plant/batch) of plants keeping four batches unsprayed. They found that a single spray of the bacterial suspention during the pick season for disease in July resulted in a satisfactory decline of the disease, the summary picture was more clear showing distinct increase of disease (3.94%) in untreared plants and with decline in disease intensity (5.21%) on treated plants.

Abhang *et al*. (2015) evaluated *in vitro* bioagents *viz., Pseudomonas fluorescens* and *Bacillus subtillis* against *X. axonopodis* pv *citri.* by turbidiometrical method. Bioagent *P. fluorescens* ($1x10^8$cell/ml.) was found effective in reducing the growth of bacteria with 0.506 OD and *B. subtilis* ($1x10^8$cell/ml.) with 0.486 OD at 96 h respectively.

Ambadkar *et al.* (2015) studied in vitro bioefficacy of two bioagents *viz., P. fluoroscens* and *B. subtilis* against *X. auxanopodis punicae* causing bacterial blight of pomogranate using inhibition zone technique. The study reveled that bioagent *P. fluoroscens* were found most effective for controlling *X. axonopodis* pv. *punicae* by forming 15.43 per cent inhibition followed by *B. subtilis* with 12.71 per cent inhibition .

Shankar *et al*. (2015) evaluated antagonistic activity of sixteen isolates of fluorescent *Pseudomonas* against *X. axonopodis* pv. *glycines* under *in vitro* condition. All the bacterial isolates significantly inhibited the growth of the test pathogen. Among the sixteen isolates, Pf 18 was found to be most significantly effective in inhibiting the growth of the pathogen and with inhibition zone of 37.33 mm, 41.00 mm and 46.00 mm after 24, 48 and 72 hrs of incubation period respectively.

Katwal (2015) reported that among different bioagents tested *in vitro* against *X. axonopodis* pv. *punicae* causing bacterial blight of pomegranate, *P. flourescens* (12.80 mm) produced maximum inhibition zone followed by *B. subtilis* (10.22 mm) and *T. viridae* (6.64 mm).

Patel *et al*. (2015) evaluated efficacy of bio control agents *viz., P. fluorescens, B. subtilis* and *T. viridae* against bacterial pathogen *X. axonopodis* pv. *punicae* causing bacterial blight in pomegranate by well diffusion and dual culture technique and concluded that, among the different antagonists in the dual culture method, *B. subtilis* and *T. viridae* were found significantly superior over *P. fluroscens* in inhibiting growth of the pathogen *X. axonopodis* pv. *punicae.*

Badiger *et al.* (2016) evaluated *in vitro* five bio-agents by inhibition zone assay method against *X. axonopodis* pv. *citri.* Among the different bio-agents studied, *B. subtilis* was effective with the inhibition zone of (16.16 mm) followed by *P. fluorescens* (14.63 mm). They also reported that, fungal bio control agents *viz., T. viride* and *T. harzianum* were found totally in effective against the pathogen.

Kumar *et al.* (2017) carried out *in vitro* evaluation of different bio-agents against *X. axonopodis* pv. *glycines*, among which *P. flourescens* recorded highest inhibitory zone (2.63 mm) followed by *B. amyloliquifaciens* and PPFM-71.

Patil *et al.* (2017) carried out *in vitro* evaluation of three bioagents against *X. oryzae* pv. *oryzae* causing bacterial leaf blight of rice. The results revealed that *T. harzianum* was found significantly superior in inhibiting the growth of pathogen (22.86 mm) followed by *P. flourescens* (17.20 mm).

Naik *et al.* (2018) tested the *in vitro* efficacy of different bioagents against *X. campestris* pv. *arecae* causing bacterial leaf stripe of arecanut, among them, *T. harzianum* (24.10 mm) was found significantly superior in inhibiting the growth of pathogen followed by *P. flourescens* (15.70 mm).

Preecha *et al*. (2018) screened *in vitro* four antagonistic bacteria *viz., B. subtilis, B. amyloliguefacien* KPS 46, and *P. pabuli,* against *X. axonopodis* pv. *citri,* causal agent of canker on *Tabtimsiam pummelo* by agar diffusion technique. Results revealed that only one antagonistic bacterium, *B. amyloliguefacien* KPS 46 expressed of secondary metabolite to inhibit *X. axonopodis* pv. *citri* of 0.14 cm

inhibition zone, while *B. subtilis* and *P. pabuli* did not express antagonism by produced secondary metabolite to inhibit this pathogen.

Daungfu *et al.* (2019) collected 170 isolates of endophytic bacteria from the six healthy *Citrus* spp. They screened all isolates, among that only 10 isolates of the endophytic bacteria could inhibit *X. citri* pv. *citri* in a dual culture technique. After screening the endophytic bacteria against *X. citri* pv. *citri*, the 10 isolates were confirmed for antagonistic effects on *X. citri* pv. *citri* by the agar well diffusion method. Results reveled that the three effective isolates, which found most effective with the highest zones of inhibition against *X. citri* pv. *citri*, were *Bacillus* LE109 (14.3 mm), *Bacillus* LE24 (11.8 mm) and *Bacillus* PO80 (10.6 mm). Where as, lowest inhibition zone was observed by *Bacillus* SO70 (4.1 mm) followed by *Bacillus* PO28 (4.3 mm).

2.6.3. Bioefficacy of plant essential oils

Kizil *et al.* (2005) studied antibacterial activity of total seven plant essential oils *viz*., Cumin (*Cuminum cyminum*),dill (*Anethum gravenoles*), coriander (*Coriandrum sativum*), anise (*Pimpinella anisum*), mint (*Mentha spicata*), hyssop (*Hyssopus officinalis*) and fennel (*Foneicum vulgare*) against four plant pathogenic bacterium *viz*., *Clavibacter michiganecse, P. syringae* and *X. campestris* pv. *malvacearum* using agar disc diffusion method. Study revealed that, coriander oil found most effective against *X. campestris* pv. *malvacearum* with inhibition zone diameter of 12 mm, followed by hyssop that is 10 mm after 72 h of inhibition time. While, cumin, anise, mint and funnel oil does not showed any inhibitory effect against *X. campestris* pv. *malvacearum.*

Kotan *et al*. (2007) evaluated inhibitory effect of 24 different essential oils in addition to pure carvacrol and thymol *in vitro*, against *X. axonopodis* pv. *vesicatoria* the causal agent of bacterial spot disease on pepper and tomato by disc diffusion method. The minimum inhibitory concentration (MIC) values were determined by using the modified agar well diffusion method at concentrations from 3,125 and 800 µl/ml. Results revealed that the pathogen was inhibited by the whole tested plant oils and pure compounds. The pure carvacrol and thymol showed the highest inhibition zone (85 mm), and MIC value was 3.125 µl/ml on the Petri plate. This zone values was the higher than inhibition zone of Streptocycline used as

positive control. They were reported that, the 24 plant samples, *Thymus canoviridis*, *Satureja hortensis*, *Melissa officinalis inodora*, *Helichrysum plicatum*, *Thymus haussknechtii*, *Thymus sipyleus* and *Thymus sipyleus rosulans* essential oils was the most active showing an inhibition zone between the range of 22-46.3 mm and a MIC of 25- 200 µl/ml.

Paret *et al.* (2010) evaluated three plant essential oils *viz.*, palmarosa (*Cymbopogon martini*), lemongrass (*C. citratus*), and eucalyptus (*Eucalyptus globulus*) against *Ralstonia solanacearum* race 4 causing bacterial wilt of ginger (*Zingiber officinale*) using culture amendment assays method. Study revealed that, *R. solanacearum* did not grew on plates amended with palmarosa oil at any concentration or lemongrass oil at 0.07 and 0.14% at 48 h after incubation. However, eucalyptus oil did not significantly prevent growth of *R. solanacearum* at any concentration except at 0.14% at 48 h.

Mikicinski *et al.* (2012) evaluated *in vitro* antibacterial activity of the total five essential oils *viz.*, lavender, sage, lemon balm, clove, and a preparation based on thyme oil against fruit diseases causing bacterial pathogens *viz.*, *Erwinia amylovora*, *X. arboricola* pv. *corylina*, *X. arboricola* pv. *juglandis*, *Agrobacterium tumefaciens* using agar plate method. Study revealed that, clove oil found most effective aginst *E. amylovora*, *X. arboricola* pv. *corylina* and *X. arboricola* pv. *juglandis* with inhibition zone 13.3mm, 11.3mm and 11.3mm respectively, followed by Biozell oil and sage oil. Least inhibition zone were found with lavender oil that is 3.3mm, 1.3mm and 1.7mm, respectively.

Lucas *et al*. (2012) studied *in vitro* antimicrobial activity of total seven plant essential oils derived from citronella, clove, cinnamon, lemongrass, eucalyptus, thyme, and tea tree each @ 0.1, 1.0, 10, and 100% concentrations in 1.0% powdered milk against *X. vesicatoria* using disc diffusion method. Study revealed that, none of the essential oils inhibited the growth of *X. vesicatoria*, at the concentration of 0.1%, while the essential oils of thyme, clove, and cinnamon partially inhibited the growth of the bacterium at the concentration of 1.0%. However, at the concentrations of 10 and 100% all of the essential oils inhibited bacterial growth.

Lee *et al.* (2012) evaluated *in vitro* total six plant essential oils, *viz*; cinnamon oil, citral, clove oil, eugenol, geraniol and limonene against *R.*

solanacearum by paper disc assay method. Study revealed that 2 µl of cinnamon oil resulted in production of a cleared zone 41.74 mm in diameter while, treatment with 5 and 10 µl increased the size of the cleared zone to 53.00 and 56.50 mm, respectively. However, at concentration of cinnamon oil to 10 µl did not enlarge the cleared zone significantly. Application of citral, clove oil, eugenol and geraniol also revealed their antibacterial activities with 2 µl in disc as 7.50, 11.50, 16.75 and 7.25mm respectively, while at concentration of 10 µl inhibition zone as 14.50, 34.00, 28.00 and 22.25mm respectively.

Rahman *et al.* (2014) evaluated *in vitro* secondary metabolites such as essential oil and pure compounds (limonin and imperatorin) from *Poncirus trifoliata* Rafin against phytopathogenic bacteria of *X.* spp. Results showed that the oil had inhibitory effect on *X. campestris* pv. *compestris* KC94-17-XCC, *X. campestris* pv. *vesicatoria* YK93-4-XCV, *X. oryzae* pv. *oryzae* KX019-XCO and *Xanthomonas* spp. SK12 with their inhibition zones and minimum inhibitory concentration (MIC) values ranging from 13.1~22.1 mm and 62.5~125 µg/ml, respectively. While, Limonin and imperatorin also had *in vitro* antibacterial potential (MIC: 15.62~62.5 µg/ml) against all the tested *Xanthomonas* spp. Further stated that, results supports the possible use of essential oil and natural compounds from *P. Trifoliata* in agriculture and agro-industries to control plant pathogenic microorganisms.

Gakuubi *et al.* (2016) evaluated antibacterial activity of essential oils of *Tagetes minuta* against three phytopathogenic bacteria *P. savastanoi* pv. *phaseolicola*, *X. axonopodis* pv. *phaseoli*, and *X. axonopodis* pv. *manihotis* by disc diffusion method. Study revealed promising antibacterial activities against the test pathogens with *P. savastanoi* pv. *phaseolicola* being the most susceptible with mean inhibition zone diameters of 41.83 and 44.83mm after 24 and 48 hours, respectively followed by *X. axonopodis* pv. *phaseoli* with inhibition zone diameter 26.83mm and 27.67mm after 24 and 48 hours, respectively. The least inhibition zone found against *X. axonopodis* pv. *Manihotis* with inhibition zone diameter 26.83mm and 23.00mm after 24 and 48 hours, respectively.

Wonni *et al.* (2016) studied effect of toatal three plant essential oils *viz.*, *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Mentha piperita* against two pathovars of *Xanthomonas oryzae* attacking rice *in vitro* by agar diffusion

method. Study revealed that all tested essential oils exhibited considerable antibacterial activity against both pathovars of *X. oryzae*. Essential oil from *C. citratus* found most effective against *X. oryzae pv. oryzae and X. oryzae pv. oryzicola* with inhibition zone diameter 34.33mm, followed by *E. camaldulensis* 12.67mm., least zone of inhibition found with *M. piperita* that is 10.22mm.

Benchouikh *et al.* (2016) evaluated total three plants' essential oils *viz.*, *C. zeylanicum, N. sativa and S. aromaticum L.* for antibacterial effect *in vitro* of on thirteen pathogenic isolates of *X. axonopodis* pv. *phaseoli* by disc-diffusion method. Result revealed that essential oils tested showed an antibacterial activity against all 13 isolates tested. The essential oil of *N. sativa* exhibited the lowest antibacterial activity in the disc-diffusion method with an inhibition zone between 10 to 12 mm of diameters. While the essential oil of *C. zeylanicum* showed a stronger antibacterial activity against these tested 13 isolates and an average antibacterial activity with *S. aromaticum*, their diameters of inhibition were respectively between 18 to 21 mm and 12 to 14 mm.

CHAPTER-III

MATERIAL AND METHODS

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3.1. Experimental site

The experiment entitled "Epidemiology and management of Mango bacterial leaf spot caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (Patel *et al.*) Robbs *et al.*" was carried out during 2018-2019, 2019-2020 and 2020-21at the Department of Plant Pathology, College of Agriculture, Parbhani and Central Nursery, VNMKV Parbhani.

The details of material used and methodology followed during the course of investigation are described here under of the methodologies to be adopted for various *in vitro* studies under present investigations are being narrated under following sub-heads.

3.2. Experimental materials

3.2.1. Disease samples

Leaves and fruits of mango exhibiting typical symptoms of bacterial leaf spot disease were collected in the paper bags from the mango orchards/ home gardens from different locations of Marathwada region during 2018-2019. The diseased samples were brought to laboratory and subjected to isolation on NA medium.

3.2.2. Equipments

Different laboratory equipments *viz.,* Autoclave, Hot air oven, Laminar airflow cabinet, BOD incubator, Refrigerator, binocular research microscope, Electronic weighing balance, pH meter, Spectrophotometer etc. available at the Department of Plant Pathology, College of Agriculture, Parbhani during the course of investigation were used as and when required.

3.2.3. Glasswares

Different types of J-seal make glass-ware *viz.*, Petri-dishes, test tubes, conical flasks, volumetric flasks, measuring cylinder, glass rods, beakers, funnel,

pipette, etc. obtained from Department of Plant Pathology, College of Agriculture, Parbhani were used.

3.2.4. Chemicals

The laboratory grade standard and pure chemicals, reagents, fungicides, culture media etc. required for the experimentation were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

Following chemicals, reagents, enzymes, primers, were utilized for studying molecular variability of the *X. campestris* pv. *mangiferaeindicae.*

A. DNA extraction

- Overnight inoculated culture of *X. campestris* pv. *mangiferaeindicae,* TES buffer (pH 8.0)
  - 50 mM Tris-HCl (pH 8.0)
  - 50 mM disodium EDTA
  - 15 mM NaCl
- *Proteinase* K 20 mg/ml
- 20% SDS
- 3M Sodium acetate
- Chloroform
- Isoamyl alcohol
- Saturated phenol
- Chilled Isopropanol
- Absolute ethanol
- 70 % ethanol
- *RNase* (2.5 mg/ml)
- Agarose
- 6X gel loading dye
- Electrophoresis buffer
- Ethidium bromide
- Sterile water

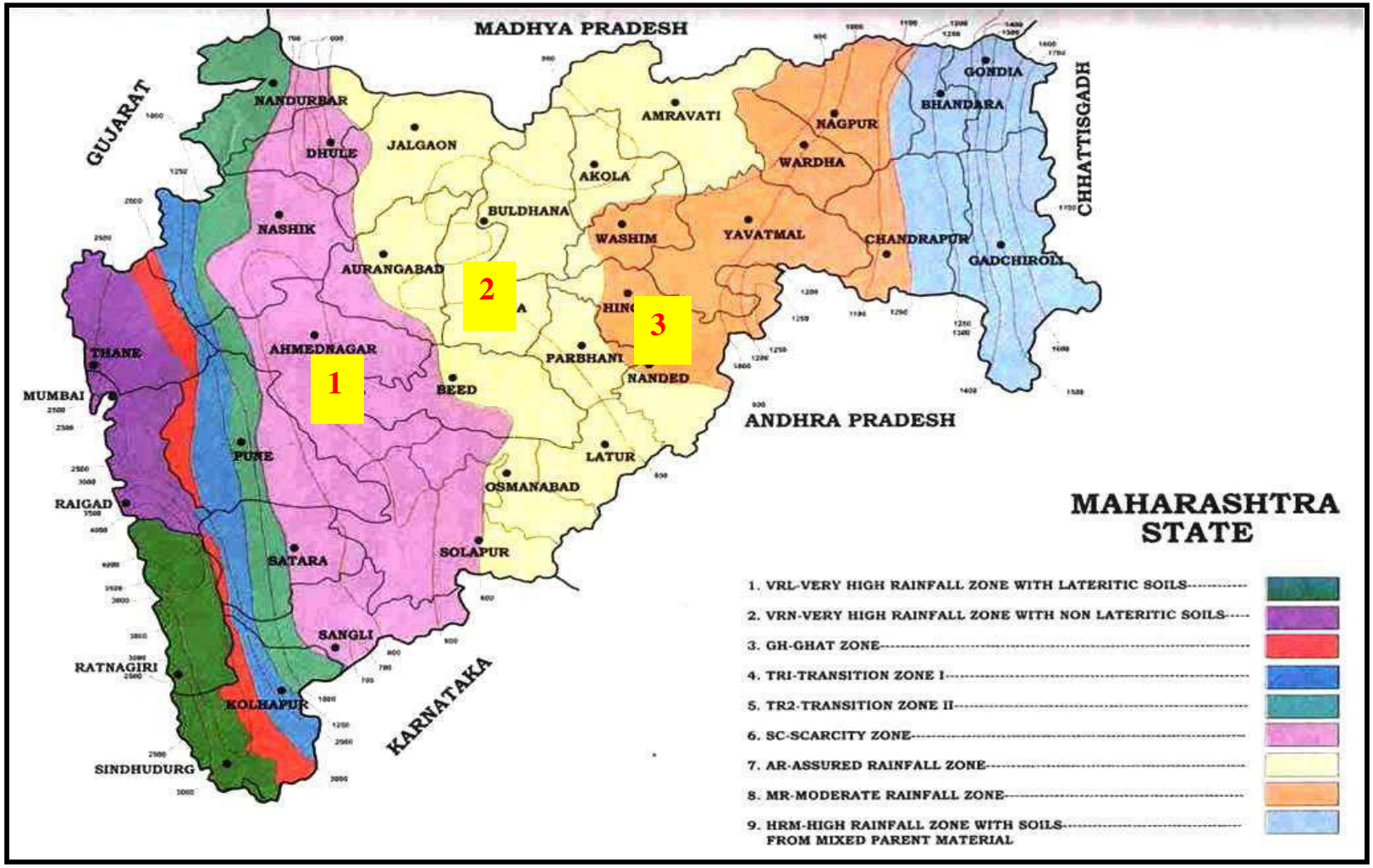


Fig. 3.1. Various agro-climatic zones of Marathwada region surveyed for mango bacterial leaf spot

Fig. 3.2. Various districts of Marathwada region surveyed for bacterial leaf spot of mango

B. RAPD analysis of *X. campestris* pv. *mangiferaeindicae* Genomic DNA

- 10X PCR buffer
- $MgCl_2$
- dNTP Mixture
- *Taq* DNA Polymerase
- Operon primer (RAPD)
- Sterile Water
- BSA, 20 mg/ml
- DMSO 100%
- 6X Gel loading dye
- 1Kb DNA ladder

C. Biochemical analysis

Benedicts quantitative reagent, anhydrous sodium carbonate, standard solution of glucose, DMSO (Dimethyl sulphoxide), 1-N Folin-Ciocalteau reagent, 20 % Sodium carbonate, Arnow's reagent, 0.05 N and 1N HCL, 1N NaOH, sulphuric acid, mercuric oxide, potassium sulphate, sodium hydroxide, boric acid and distilled water.

The various kinds of materials *viz.*, fungicides, antibiotics, essential oils, chemicals, biocontrol agents, glass-wares, culture media and other miscellaneous items required for conducting present studies were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

3.2.5. Seedlings

Seedlings of mango cultivars *viz.,* Local, Kesar, Dasheri, Neelam, Amrapali and Alphanso were used for various lab and pot culture experiments from Central Nursery Scheme, VNMKV Parbhani.

3.2.6. Antibiotics

The following 6 antibiotics and 3 antibacterial fungicides were used for *in vitro* (Plate culture and pot culture) experiments conducted during present studies.

Table A. List of antibiotics

Sr. No.	Common name	Trade Name	Manufacture	Chemical name
1.	Streptocycline	Streptocycline	Hindustan Antibiotics Ltd. Mumbai	Streptomycin sulphate 90%w/w + Tetracycline hydrochloride 10%
2.	Bacterinashak	Bacterinashak	Indofil Chemicals Company Ltd.	2-Bromo 2-Nitro propane 1, 3-diol
3.	Biomycin	Biomycin	Biostadt India Limited	Kasugamycin 3% S. L.
4.	Plantomycin	Plantomycin	Aries agro Ltd, Mumbai	Streptomycin sulphate 9% w/w + Tetracyclin hydrochloride 1% w/w
5.	Conika	Conika	Dhanuka agritech Ltd.	Kasugamycin 5% + Copper oxycloride 45% wp
6.	Bacterinol	Bacterinol	Bronopol-Royal crop science (India) Panipat, India	95% 2-Bromo 2-Nitropropane-1, 3-diol

3.2.7. Fungicides

Table B. List of fungicides

Sr. No.	Common name	Trade Name	Manufacture	Chemical name
1	Carbendazim	Bavistin	BASF India Ltd, Mumbai, (M.S.)	Carbendazim 50 WP
2	Bordeaux mixture	-	-	Bordeaux mixture 1%
3	Mancozeb	Dithane M-45	Indofil Chemical Co. Ltd., Mumbai, (M.S.)	Mancozeb 75 WP
4	Cyamoxanil + Mancozeb	Curzet	E. I. Dupont India Pvt. Ltd., Mumbai, (M.S.)	Cyamoxanil + Mancozeb 8/64 WP
5	Copper oxychloride	Blitox	Syngenta Ltd., Mumbai	Copper oxychloride 50% WP
6	Copper hydroxide	Koside	Green crop International Ltd. Pune	Copper hydroxide 77% WP
7	Clorothalonil	Kavach	Syngenta Ltd, Mumbai, (M.S.)	Clorothalonil 75%WP

3.2.8. Biocontrol agents

Pure cultures of biocontrol agents *viz*., *Trichoderma viride*, *T. harzianum, Gliocladium virens*, *Verticillium lecanii* and *Pseudomonas fluorescens, Methylobacteria, Bacillus substilis, P. striata* were obtained from the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani; maintained and multiplied on appropriate culture media and used for further studies.

3.2.9. Essential oils

Table C. List of essential oils

Sr. No.	Common Name	Trade Name	Manufacture	Quantity (ml)
1	Neem oil	Neem oil	Health Vit	30
2	Citronella oil	Citronella Essential oil	Spice herbal & Amenities Pvt. Ltd., Delhi.	15
3	Clove oil	Clove oil	Gandhi herbal India Pvt. Ltd., Guradiya Dwda, Mandsaur (M. P.) India	2
4	Cinnamon oil	Oil Darchini	Fame drugs. Pvt. Ltd. Meerut (U. P.)	5
5	Eucalyptus oil	Nilgiri oil	Gandhi herbal India Pvt. Ltd., Guradiya Dwda, Mandsaur (M. P.) India	7
6	Mentha oil	Mint Essential oil	Spice herbal & Amenities Pvt. Ltd. Delhi.	15
7	Garlic oil	Garlic oil	Yogesgh Pharmacy,	10
8	Ginger oil	Ginger oil	Vivaa Urban Essentials, Delhi	15
9	Piper oil	Black pepper Essential oil	Pacific computec Pvt. Ltd. Gurugram	10

3.2.10. Miscellaneous

Plant protection appliances, inoculation needle, tuberculin syringe, forceps, blotter paper, paper bags, polythene bags, spirit lamp, mercuric chloride, labels, scales, sand, soil, FYM, screen house etc. available at the Department of Plant Pathology, VNMKV, Parbhani were used.

3.3. Methodology

The details of the methodologies adopted for various *in vitro* studies under present investigations is narrated under following sub-heads.

3.3.1. General laboratory procedure

All the glassware's were kept for 24 hrs in cleaning solution containing 60.0gm of potassium dichromate ($K_2Cr_2O_7$), 60.0 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water and were washed with soap powder followed by washing in running tap water and then finally rinsed with distilled water.

3.3.2. Sterilization

All the glassware's were sterilized in hot air oven at 160^0 C for two hrs. Sterilization of both solid and liquid media was achieved by autoclaving at 1.1 kg/cm^2 (121.6^0C) pressure for 20 minutes for all the laboratory studies.

3.4. Collection of diseased specimens

The mango plant sample, leaves, fruits, branches showing typical symptoms of bacterial leaf spot was collected in paper bags, from various orchards / gardens located in three agro-climatic zones of Marathwada region, subscribed with relevant information, be brought to the laboratory and subjected aseptically to isolations on nutrient agar medium.

3.4.1. Symptomatology

During collection of the mango naturally bacterial leaf spot diseased plant specimens (leaves, fruits and branches) were diagnosed for manifestation of bacterial leaf spot symptoms. Also in pot culture experiments, bacterial leaf spot symptomatology was studied.

3.4.2. Isolation of the bacterium

The bacterial leaf spot infected mango plant specimens (leaves, fruits and branches) collected from mango orchards distributed in various agro- climatic zones of Marathwada region, were subjected first to ooze test to confirm association of the bacterium and then subjected to isolations on nutrient agar (NA) medium, by employing standard procedure. Upon completion of the incubation period, single colonies of the bacterium develop on NA was picked up aseptically, transferred on to fresh NA Petri plates and incubated 28 ± 2^0 C, to obtained pure cultures of the bacterium isolates/strains. Applying same procedure, a total of 35 isolates of *X. campestris* pv. *mangiferaeindicae* were isolated. Pure cultures of the test bacterium

strains were assigned the nomenclature, by considering the agro climatic zones and / or the mango plants from which day isolated. Pure culture thus obtained was preserved in refrigerator for further studies. Details of the codes / nomenclature assigned to the 35 test isolates of *X. campestris* pv. *mangiferaeindicae* are as given below:

Table D. Description of 35 isolates collected from different agro climatic zones of Marathwada region

Sr. No.	Agro climatic zones	District	Location	Isolate name
1	SC-Scarcity zone	Aurangabad	Gangapur	Xcm1
2		Beed	Anandwadi	Xcm2
3			Belgaon	Xcm3
4		Osmanabad	Ashta	Xcm4
5	AR- Assured rainfall zone	Aurangabad	Pachod	Xcm5
6			Paithan	Xcm6
7		Jalna	Antarweli	Xcm7
8			Jalna	Xcm8
9			Mangrul	Xcm9
10		Beed	Girwali	Xcm10
11			Ashti	Xcm11
12			Majalgaon	Xcm12
13			Keij	Xcm13
14		Osmanabad	Dhangarwadi	Xcm14
15			Kalamb	Xcm15
16			Alni	Xcm16
17		Latur	Dagadwadi	Xcm17
18			Hipparga	Xcm18
19			Latur	Xcm19
20			Madansuri	Xcm20
21		Parbhani	Kolha	Xcm21
22			Bori	Xcm22
23			Zari	Xcm23
24			Renapur	Xcm24
25			Makhni	Xcm25
26		Nanded	Kandhar	Xcm26
27			Markhel	Xcm27
28			Loha	Xcm28
29		Hingoli	Yeldari	Xcm29
30	MR- Moderate rainfall zone	Nanded	Mudkhed	Xcm30
31			Vishnupuri	Xcm31
32			Himayatnagar	Xcm32
33		Hingoli	Aundha	Xcm33
34			Hingoli	Xcm34
35			Basmat	Xcm35

3.4.3. The pathogen studies

3.4.3.1. Pathogenicity test

3.4.3.1.1. Standardization of inoculation methods

The pathogenecity test was performed with pure culture of the bacterial pathogen isolated from diseased samples affected with *X. campestris* pv. *mangiferaeindicae*. Three different methods *viz.,* pin-prick method, infiltration method and spray inoculation method were used for confirming the pathogenecity of the causal bacterium. Fresh growth of 72 hrs. of *X. campestris* pv. *mangiferaeindicae* on nutrient agar plate or test tube was used for using the pathogenecity. The susceptible cv. Kesar of mango was used for this study.

Inoculation methods

a) Pin-prick method

Lower surface of leaves was pin-pricked with sterilized needle in multiple (10-15 numbers) points. Bacterial inoculums (2 x 10^8 cfu/ml population) was placed on the pricked area by placing a drop of cell suspension and through cotton swab which was pre soaked in inoculum.

b) Infiltration method

Bacterial inoculum was taken in an disposable injection syringe of 0.5ml capacity. Care was taken that no air bubble was trapped in the suspension. The needle of syringe was placed on lower surface of the leaf, particularly at interveinal region and gently inoculum was injected. The leaf was firmly hold by providing support on the opposite side at the point of injection with the help of index finger (left hand), while the thumb (left hand) was placed on the lower side above the mouth of the needle. The inoculum was infiltrated slowly by pressing the syringe with the thumb (right hand). Bigger wounds were avoided at the point of injection. Plants were incubated in humid condition. After infiltration, plants were periodically monitored and observed for hypersensitive and susceptible reaction. The hypersensitive reaction appeared within 8 - 24 h of infiltration, while susceptible reaction required 72 - 96 h depending on pathogens virulence level, etc.

c) Spray Inoculation Method

Healthy susceptible host plants were sprayed on the foliage with the help of hand sprayer. Sprayed plants were incubated in humid condition and turgidity of leaf was maintained with the help of humidifier.

Re-isolation

The pathogen was re-isolated from the artificially inoculated plant under poly house condition. The isolations yielded a yellow culture of *X. campestris* pv. *mangiferaeindicae* on nutrient agar medium.

3.4.3.1.2. Attached leaf assay (Francis *et al.* 2010).

Mature leaves on greenhouse seedlings of mango of the same type used for detached leaf assay were inoculated using needleless syringe. Test bacterial inoculum (as described under detached leaf assay) was infiltered by pressing needleless syringe to produce a zone of water-soaked tissue about 6mm in diameter. Three injection infiltrations were performed on each side of the midvein. Three leaves were inoculated per plant, and three plants were inoculated per assay. The inoculated shoots were covered with a plastic bag for 24 h to maintain high humidity conductive for bacterial growth in the leaves. Developments of symptoms on leaves were evaluated periodically up to 21 days post inoculation.

3.4.3.1.3. Detached leaf assay (Yin *et al*. 2011**)**

A single bacterial colony was collected from the test bacteria cultured on NA medium and inoculated in NA broth for mass multiplication. The resultant culture was kept at 28^0C and shaken overnight at 200 rpm, which was collected and resuspened in the medium to the final concentration of 10^8cfu/ml before inoculation. Two third to fully expanded, mature leaves from mango susceptible year old cultivar of Kesar seedlings, grown in green house was detached and brought in to laboratory. Mango leaves were first washed with distilled water and then subjected to inoculation on abaxial side using an insect pin (0.5mm in diameter). Four inoculation sites (each composed of 5 pricks) were made on both surface of midvein, onto which an aliquot of 10µl of the bacterial suspension was dropped with the help of micropipette. Following the inoculation, the leaves were placed above wet filter paper in Petri dish,

which were sealed with parafilm to sustain high humidity. Two leaves were taken for inoculation of per isolate. The petri dishes were maintained at 28^0C in incubator for up to 21 days. At various time points, occurrence of symptoms was scored.

3.4.4. Pathogenic variability

Pathogenic variability of different isolates collected from different locations were studied by using pathogenicity tests as described under 3.4.3.1.1 (b)

3.4.5. Morpho- cultural characterization

Morpho-cultural characters of *Xanthomonas campestris* pv. *mangiferaeindicae* pathogen will be studied by microscopic and visual observation of Morpho-cultural characters, *viz.* cell shape, flagellation, colony edge, elevation, Pigmentation and surface appearance.

3.4.6. Biochemical characterization

Biochemical characters of *Xanthomonas campestris* pv. *mangiferaeindicae* pathogen were studied by subjecting the bacterial isolates to various biochemical tests as follows:

a. Gram Staining

The Gram-reaction of each isolate was determined following the staining procedure in Schaad *et al.* (2001). First a loop full of the bacterium suspension was smeared on clean glass slide, air fixed by gentle heating on flame of the spirit lamp. Aqueous Crystal violet solution (0.5%) was spread over this smear for 30 second and then washed with running tap water for a minute; this stained smear was later flooded with Grams iodine solution for one minute and rinsed in tap water. Later decolorized with 95% of ethanol until colour runoff, washed with water and treated with Safranin as counter stain about 10 seconds, washed with water, air/blot dried and observed under research microscope (make:- Olympus) at 100X using oil immersion technique.

b. Catalase oxidation test

A loop full of 24-28 hrs old culture of test bacterium was placed on the clean glass slide, and to this a drop of 3% hydrogen peroxide (H_2O_2) was mixed and allowed to react for few minutes and observed for the production of gas bubbles.

c. KOH test (Potassium hydroxide)

A drop of 3 per cent potassium hydroxide was placed on clean glass slide and to this 48 hr old bacterial culture was mixed with clean inoculation loop and stirred for 10 sec and observed for slime threads. When raised the wire loop, if strands of viscid material seen, then the bacterium is gram negative.

d. Starch hydrolysis

For each hydrolysis test 20g Nutrient Agar (NA) was added to 80 ml of water and dissolved by successive heating and stirring similarly two gram starch was then thoroughly dissolved in 10 ml distilled water separately and added to hot molten agar with through stirring. Amount of 100 ml of this basal medium was then transferred to conical flask (250 ml) and autoclaved at 115^0C for duration of 10 minutes. The medium was then poured into Petri plates. The plates were then inoculated with individual isolate aseptically, labeled and sealed to avoid chances of contamination. These plates were then incubated in upside down position at 27^0C for 7 days. After scraping bacterial growth to each plate Lugol's iodine was added which was prepared by mixing 1g iodine and 2 g potassium iodide in 300 ml distilled water, stirred for until dissolved completely. The appearance of cleared zones around the colonies was indicative of presence or absence of starch hydrolysis. (Rafi *et. al.* 2013)

e. Gelatin liquefaction

Nutrient gelatin medium (Peptone 10.0 g, beef extract 5.0 g, gelatin 20.0 g, water 1000 ml and pH 7.0) was used for this test. The medium was taken in the tubes and sterilized at 15 lbs pressure for 20 min. Allowed the tubes to cool and stand at 20˚C for two days to check the sterility. Later, inoculated these gelatin columns by stabbing a straight inoculation needle charged with the test bacterium. All the tubes were incubated at 27˚C. They were observed for liquefaction of the gel column and if it remains liquid, the result is positive.

f. Casein hydrolysis

The ability of the bacterium to degrade the protein casein by producing proteolytic exo-enzymes was tested by growing the isolates on milk agar plates (Skim milk powder 100g, peptone 5 g, agar 5 g, distilled water 1000 ml and pH 7.2 was maintained). The medium was sterilized (at 121˚C for 15 minutes) in autoclave. Observation on formation of zone around the bacterium was recorded.

g. Xanthum gum production test

The SPA medium recommended (Sucrose 20 g, peptone 5 g, agar 15 g, K_2HPO_4 0.5 g, $M_gSO_47H_2O$ 0.25g, and distilled water 1000 ml) for xanthum gum production tests was used. A well dried SPA medium was streaked with colonies of test bacterium and incubated at 25-27˚C for 24 h. After 2-3 days, the plates were examined for the production of xanthum gum.

h. Anaerobic growth test

5 ml Basal medium [Peptone: 2g; NaCl: 5g; Agar: 0.3g; KH_2PO_4: 0.3g; bromothymol blue: (3 g) in 1% aques solution, 1 lit final volume] was poured into test tubes and sterilized at 121^0C for 20 minutes. An amount 0.5 ml 10% glucose suspension was added to each tube aseptically. For each isolate two test tubes were inoculated. One of the tubes was sealed with paraffin, and incubated at 27^0C. Anaerobic growth was noticed if color change occurred from blue to yellow. (Rafi *et al.* 2013)

i. Tetrazolium salt tolerance test

Nutrient agar was prepared and dispensed in 100 ml flasks and sterilized at 121^0C for 15 minutes. Aqueous 1% TTC solution was aliquoted (filter-sterilized) to the molten agar at 55^0C to give a concentration of 0.02%. Similarly, 0.1% concentration was also prepared. The medium was then dispensed in plates and inoculum was added to the medium held at two different concentrations whereas nutrient agar alone served as control. Presence or absence of growth was recorded since most *Xanthomonads* are inhibited at 0.02% and completely inhibited at 0.1% concentration of TTC. (Mubeen *et al.,* 2015, Rafi *et al*., 2013; Rahman *et al.,* 2010).

3.4.7. Molecular characterization

Random amplified polymorphic (RAPD) DNA analysis was used to detect molecular / genetic variations among the isolates of *X. campestris* pv. *mangiferaeindicae*. Standard protocols were used for the isolation of DNA and RAPD analysis. Services provided by Quencher biotech. Pvt. Ltd. were used for purpose of same.

3.4.7.1. Extraction of genomic DNA

The extraction of genomic DNA from isolated of *X. campestris* pv. *mangiferaeindicae* was carried out by following a protocol described by Adachi and Takashi (2002) with some modifications. Three ml NA broth culture was centrifuged for 5 min at 7,000 rpm. The pellet of isolates of *X. campestris* pv. *mangiferaeindicae* were washed with 0.7% NaCl and TEN (Tris HCl 50 mM, pH-7.5, EDTA 10 mM, pH-8.0, and NaCl 150 mM) buffer, vortexed and centrifuged at 7,000 rpm for 5 min. The bacterial cells were resuspened in 500 µl of *proteinase* K solution (150 µg /µl in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0) and kept at 56^{o}C for 15 min, followed by incubation at 80^{o}C for 15 min to denature the *proteinase* K, further suspension was centrifuged for 5 min at 13000 rpm. The supernant was transferred to a new microfuge tube and treated with *RNase* A (100 µg/ ml) for 30 min at 37^{o}C. Further the reaction mixture was subjected for DNA precipitation extraction with phenol: chloroform: isoamylalcohol (25:24:1) followed by addition of 0.1 volume of ammonium acetate and 2 volumes of chilled ethanol. The DNA pellet was washed with 70% ethanol and resuspened in 50 µl sterile water. The DNA samples were quantified by using Spectrophotometer at 260 nm wavelength. Similarly the quality of DNA was assessed by separating on 0.8 % agarose gel.

3.4.7.2. Standardization of RAPD Protocol

The RAPD-PCR protocol described by Williams *et al.,* (1990) was used with some modifications, to produce RAPD fingerprinting profile of eight strains of *Xanthomonas*. The PCR amplification reaction was optimized by varying concentration of PCR components. Amplification reaction was carried out in 25 µl reaction mixture containing 30 ng of genomic DNA, 1X PCR buffer, 1.5 mM MgCl 2 , 0.25 mM dNTPs, 0.4 µM primers and 1.25 U of Taq. DNA polymerase. PCR

amplification was performed in master cycler gradient Eppendorf PCR Machine. The program consisted of an initial denaturation at 94^0 C for 4 min, followed by 39 cycles of 94^0C for 1 min. 35^0C for 1 min and 72^0C for 1.5 min, followed by final extension at 72^0 C for 10 min. Finally a product was hold at 4^0C. PCR amplified product was separated by electrophoresis in 1.2 % agarose gel in 1X TAE buffer stained with Ethidium bromide and visualized under gel documentation system.

3.4.7.3. Confirmation of *Xanthomonas* strains using PCR

a) PCR Reaction

The eight strains of *X. campestris* pv. *mangiferaeindicae* was confirmed by using genus specific diagnostic primer. The PCR protocol used for preparation of reaction mixtures and thermal cycler programme for PCR based detection is described in Table No and No respectively.

Table E. PCR components used in preparation of reaction mixture

PCR Components	Required Concentration	Volume/reaction
PCR Buffer (10X)	1X	2.5 µl
$MgCl_2$ (25 mM)	1.5 mM	1.5 µl
10 mM dNTP mix.	200 µM	2.0 µl
Forward primer (10 µM)	0.4 µM	1. µl
Reverse primer (10 µM)	0.4 µM	1. µl
Taq DNA Polymerase (3 U/µl)	1.25 U	0.5 µl
Template DNA	30 ng	1.0 µl
Nuclease free water		15.5 µl
Total		**25 µl**

Master mixture (24.0 µl) containing all the above reactants, except template DNA were dispensed in autoclaved PCR tubes (0.2 ml). Genomic DNA of each isolates of *X. campestris* pv. *mangiferaeindicae* was added to the individual tubes containing the master mixture. The contents of each tube were mixed by tapping with fingers followed by a brief spin to collect contents at the bottom of the tube. The tubes were placed in thermo cycler and the following PCR protocol used for the confirmation of species of *Xanthomonas* by using genus-specific primer. PCR was performed in 25 µl reaction mixture (Table F).

Table F. Standardized PCR protocols used in confirmative test of strains of *X. campestris* pv. *mangiferaeindicae*

Sr. No.	Steps	Temperature		Time
1.	Initial Denaturation	94^0C		4 min
2.	Denaturation	94^0C	29 cycles	45 sec
3.	Annealing	56^0C		45 sec
4.	Primer Extension	72^0C		1 min
5.	Final extension	72^0C		5 min
6.	Hold	4^0C		Forever

b) Resolution of amplified product

Amplified PCR product was resolved on 1.2 % agarose gel in Tris-Acetate-EDTA (TAE) buffer, stained with ethidium bromide and visualized under Gel Documentation System.

3.4.7.3. Determination of genetic variability by using RAPD analysis

a) PCR Reaction

The PCR protocol for RAPD reaction was optimized with various PCR components (Table G) and thermal cycler programme (Table H).

Table G. PCR Components used for genetic diversity analysis of strains of *Xanthomonas.*

PCR Components	Required Concentration	Volume/reaction
PCR Buffer (10X)	1X	2.5 µl
$MgCl_2$ (25 mM)	1.5 mM	1.5 µl
dNTP mix. (10 mM)	200 µM	2.0 µl
Primer (10 µM)	0.4 µM	1.0 µl
Taq DNA Polymerase (3 U/µl)	1.25 U	0.5 µl
Template DNA	30 ng	1.0 µl
Nuclease free water		16.5 µl
Total		**25 µl**

Random primers *viz.,* OPA, OPB and OPC series (Operon Technologies, USA) were used for RAPD analysis of eight strains of *X. campestris*

pv. *mangiferaeindicae* species described in Table No. Master mixture (24.0 µl) containing all the above reactants, except template DNA were dispensed in autoclaved PCR tubes (0.2 ml). Genomic DNA of each strain of *Xanthomonas* species was added to the individual tubes containing the master mixture. The contents of each tube were mixed by tapping with fingers followed by a brief spin to collect contents at the bottom of the tube. The tubes were placed in Thermocycler and subjected to PCR according to the protocol adopted below (Table H).

Table H. Standardized PCR protocols for amplification of DNA

Sr. No.	Steps	Temperature		Time
1.	Initial Denaturation	94^0C		4 min
2.	Denaturation	94^0C	39 cycles	1 min
3.	Annealing	35^0C		1 min
4.	Primer Extension	72^0C		1.5 min
5.	Final extension	72^0C		10 min
6.	Hold	4^0C		Forever

b) Resolution of amplified product

The amplified RAPD PCR product was separated on 1.2 % agarose gel, stained with ethidium bromide and visualized under Gel Documentation System. The polymorphism was detected by comparing RAPD fingerprinting pattern of all *X. campestris* pv. *mangiferaeindicae* strains.

3.4.7.4. Data analysis

3.4.7.4. 1. Scoring of amplified fragments

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

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

Data matrices were generated and used to plot dendrogram by using Jaccard's similarity coefficient by using software NTSYS-pc.2.02i.

3.4.8. Effect of different age of leaf on disease development

The effect of eight isolated on detached leaf of three different stages were tested in controlled conditions. The three stages *viz.,* young, mid age and old leaves were tested against eight isolates. The leaves were kept on moist butter paper in petri plate. Eight bacterial isolates were multiplied on nutrient broth in a flask separately and incubated at 28^0 C for three days. The leaves were injured with the help of inoculating needle and the broth was poured on injured leaves. After inoculation the leaves were kept in BOD at 28^0 C. The observations were made on the development of symptoms on three different stages of leaf.

3.5. Host range

The host range studies were conducted using nine fruit crop species *viz.,* lemon, sweet lime, pomegranate, jamun, fig, almond, guava, custard apple and mango. The ability of pathogen to infect the different host crop plants was tested by inoculating the pathogen to the leaves of crops under controlled conditions. The leaves of fruit crop was kept on moist butter paper in petri plates. The eight bacterial isolates were multiplied on nutrient broth in flask separately and incubated at 28^0 C for three days. The concentration of bacterial cells was adjusted to 10×10^8 cfu/ml of water in spectrophotometer. The leaves were injured slightly with inoculating needle. The bacterial inoculum of eight of isolates was sprayed separately on the surface of injured leaves. After inoculation the leaves were kept in BOD at 28^0 C temp. The observations were made on the development of dark brown lesions on the leaves.

3.6. Disease epidemiology

3.6.1. Survival of *X. campestris* pv. *mangiferaeindicae*

A study was undertaken to know survival period of *X. campestris* pv. *mangiferaeindicae.* Affected leaves, twigs and bark were collected from infected orchards in the month of August- September, 2018 and stored at room temperature in paper bags and used for isolation on nutrient agar media by streak plate method at an interval of 15 days for further studies on survival of the pathogen on plant parts.

3.6.2. Effect of Temperature and Leaf Wetness Duration on the disease development in detached leaves

Leaves were first washed with sterilized distilled water to remove the dust particles and other materials adhering to the surface of the leaves. These leaves were then put in the sterilized petriplates of 6 inch in size laid with blotting paper. Inoculum of 4.5×10^6 cfu/ml was prepared and injected into the petiole of the leaves with sterilized syringe. The petriplates containing leaves were regularly moistened with sterilized distilled water. Observations were recorded after 3, 6, 12, 24, 48 and 72 hours for the symptoms appearance.

Apparent infection rate

$$r = \frac{2.3}{t_2 - t_1} [\text{Log}\ (X_2 / 1\text{-}X_2) - \text{Log}\ (X_1 / 1\text{-}X_1)]$$

(Vander Plank, 1963)

Area Under Disease Progress Curve (AUDPC)

$$\text{AUDPC} = \sum_{Ni-1} Yi\text{-}Yi+1 \ / \ (t_i+1\text{-}t_i)$$

(Shaner and Finney, 1977)

3.6.3. Role of weather parameters on bacterial leaf spot development in Mango

As per the literature, it is known that bacterial infection in mango get aggravated with the prevalence of high temperature, humidity, coupled with rainfall. Present investigation was aimed at studying the effect of these environmental factors in relation to the disease. A field trial was taken up. Susceptible variety Kesar was selected for this study. The plants were selected for the observations on the incidence and severity of bacterial leaf spot on both twigs and leaves at weekly interval right from initial appearance of symptoms. Corresponding, prevailing weather parameters during standard meteorological weeks from 14 to 37 th SMW such as maximum and minimum temperature, rainfall and relative humidity was also recorded. Data was analyzed statistically and correlated with weather parameters.

3.7. Disease management strategies

3.7.1. *In vitro* evaluation of antibiotics

Antibiotics (each @ recommended field dosages, 50% of recommended and 125% of recommended), were evaluated alone and with combination of fungicides *in vitro* by applying inhibition zone assay (Kirby-Baur 1950) method as described by (Raju *et al.* 2012) and using Nutrient Agar (NA) as basal medium. The mass multiplied broth culture of test bacterium ($2x10^8$cfu/ml) was seeded to autoclaved and cooled (45^0C) Nutrient agar medium, mixed thoroughly, poured into sterilized glass Petri plates and allowed to solidify.

The test antibiotics solutions of various concentrations (each @ recommended field dosages, 50% of recommended and 125% of recommended dose) in distilled water was prepared separately. The filter paper discs (Whatman No.42) of 5 mm in diameter was soaked separately in the respective chemical solutions for 5-10 minutes and put in the center onto solidified bacterium seeded NA medium in Petri plates. The inoculated plates were kept in the refrigerator at 4^0 C for 4 hours to allow diffusion of the chemical into NA medium. Untreated control plates filled with the test bacterium seeded NA and inoculated with filter paper disc soaked in distilled water was maintained. Then the plates were incubated at 28 ± 2^0C, in BOD incubator.

Experimental details:

Design : CRD

Replications : Three

Treatments : Eight

Table I. Treatment details:

Tr. No.	Treatment details	RD (ppm)	50% RD (ppm)	125%RD (ppm)
T_1	Streptocycline sulphate 90%w/w + Tetracycline hydrochloride 10% (Streptocycline)	100	50	125
T_2	2-Bromo 2-Nitro propane 1,3-diol (Bacterinashak)	100	50	125
T_3	Kasugamycin (Biomycin)	300	150	375
T_4	Streptomycin sulphate 9% w/w + Tetracycline hydrochloride 1% w/w (Plantomycin)	100	50	125

T_5	Kasugamycin 5% + Copper oxycloride 45% wp (Conika)	2000	1000	2250
T_6	Bacterinol (95% 2-Bromo 2-Nitropropane-1, 3 diol)	100	50	125
T_7	Bacterinol + Copper oxycloride	100 + 3000	50 + 1500	125 + 3125
T_8	Control (untreated)	--	--	--

Observation on inhibition zone around the filter paper disc was recorded at 24 hrs intervals and continued till 120 hrs after incubation and the average zone of inhibition was calculated.

3.7.2. *In vitro* evaluation of fungicides

Antibacterial fungicides (each @ recommended field dosages, 50% of recommended and 125% of recommended), were evaluated *in vitro* by applying inhibition zone assay (Kirby-Baur 1950) method as described by (Raju *et al.* 2012) and using Nutrient Agar (NA) as basal medium. The mass multiplied broth culture of test bacterium ($2x10^8$cfu/ml) was seeded to autoclaved and cooled (45^0C) Nutrient agar medium, mixed thoroughly, poured into sterilized glass petri plates and allowed to solidify.

The test fungicide solutions of various concentrations (each @ 1000, 2000 and 3000 ppm) in distilled sterilized water were prepared separately. The filter paper discs (Whatman No.42) of 5 mm in diameter were soaked separately in the respective chemical solutions for 5-10 minutes and put in the center onto solidified bacterium seeded NA medium in Petri plates. The inoculated plates were kept in the refrigerator at 4^0C for 4 hours to allow diffusion of the chemical into NA medium. Untreated control plates filled with the test bacterium seeded NA and inoculated with filter paper disc soaked in distilled water were maintained. Then the plates were incubated at 28 ± 2^0C, in BOD incubator.

Experimental details:

Design : CRD

Replications : Three

Treatments : Eight

Table J. Treatment details

Tr. No.	Chemical name	RD (ppm)	50%RD (ppm)	125%RD (ppm)
T_1	Carbendazim 50 WP	100	500	1250
T_2	Bordeaux mixture 1%	1%	0.5%	1.25%
T_3	Mancozeb 75 WP	2500	1250	3125
T_4	Cyamoxanil 8%+ Mancozeb 64% WP	2500	1250	3125
T_5	Copper oxychloride 50% WP	3000	1500	3750
T_6	Copper hydroxide 77% WP	2500	1250	3125
T_7	Clorothalonil 75%WP	2000	1000	2500
T_8	Control (untreated)	-	-	-

Observation on inhibition zone around the filter paper disc was recorded at 24 hrs intervals and continued till 120 hrs after incubation and the average zone of inhibition was calculated.

3.7.3. *In vitro* evaluation of plant essential oils

Antibacterial activity of essential oils was evaluated (Each @ 1000 and 2000 ppm) using disc diffusion method (Kirby-Baur 1950) as described by (Gakuubi *et al.*2016) also known as Kirby-Bauer antimicrobial susceptibility test. Bacterial suspension (10^8cfu/ml) prepared from an overnight culture was uniformly spread on Petri plates (9 cm in diameter) containing using a sterile L-shaped glass rod. Sterile (Whatman No.42) filter paper discs of 5 mm in diameter each were impregnated separately in the respective concentrations of essential oils in a laminar air flow cabinet. Prior to that different concentration of essential oils were blended with one percent tween 80 solution for mixing of oils in water while making different concentrations and vortexed vigorously on vortex mixture to break lipofication bond between water and oils. The discs were then aseptically placed at the center of the inoculated culture plates using sterile forceps. Distilled water was used as a negative control. The plates were refrigerated at 4^0C for 2 hours to allow the essential oils to diffuse into the agar medium and finally incubated upside down at 27 ± 2^0C temperature.

Experimental details:

Design : CRD

Replications : Three

Treatments : Ten

Treatment details

T_1 : Neem oil

T_2 : Citronella oil

T_3 : Clove oil

T_4 : Cinnamon oil

T_5 : Eucalyptus oil

T_6 : Mentha oil

T_7 : Garlic oil

T_8 : Ginger oil

T_9 : Piper oil

T_{10} : Control (untreated)

Observation on inhibition zone around the filter paper disc was recorded at 24 hrs intervals and continued till 120 hrs after incubation and the average zone of inhibition was calculated.

3.7.4. *In vitro* evaluation of bioagents

The biocontrol agents *viz., Bacillus subtilis, Psuedomonas fluorescens, P. striata, Trichoderma harzianum, T. viride, T. hamatum, T. virens, Verticillium lecanii* etc. alone were evaluated for their efficacy against the growth of *X. campestris* pv. *mangiferaeindicae,* by inhibition zone assay method. (Raju *et al,* 2012).

A heavy suspension (3 days old) of *X. campestris* pv. *mangiferaeindicae* 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 (bacterium) organism will be 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 will be placed in the center of the plates. The inoculated plates will be then

incubated at 30^0 C for 72 hours. Observations will be recorded for the zone of inhibition produced by antagonistic micro-organisms around the growth of the pathogen.

Experimental details:

Design : CRD
Replications : Three
Treatments : Eight

Treatment details:

T_1 : *Bacillus subtilis*
T_2 : *Psuedomonas flourescens*
T_3 : *P. striata*
T_4 : *Trichoderma harzianum*
T_5 : *T. viride*
T_6 : *Verticillium lecanii*
T_7 : *T. hamatum*
T_8 : *T. virens*
T_9 : Control (untreated)

Observation on inhibition zone around the disc was recorded at 24 hrs intervals and continued till 120 hrs after incubation and the average zone of inhibition was calculated.

3.7.5. Disease management in green house (Pot culture)

Those antibiotics, antibacterial fungicides and antagonists found most effective against the *X. campestris* pv. *mangiferaeindicae* during present *in vitro* studies were selected and assessed for integrated management of bacterial leaf spot disease of mango. The earthen pots (60 cm dia.) filled with potting mixture of soil: sand: FYM (2:1:1) planted with mango seedlings were used. One year old seedlings with uniform vigour and stem diameter (approximately 1 cm) was cut back to allow one dominant shoot to develop. Eight leaves per plant as well as four areas per leaf were selected for puncturing (pin prick method). In each area, 20 wounds were made and the upper leaf surfaces immediately sprayed with mixture of *X. campestris* pv.

mangiferaeindicae strains. Inoculated seedlings immediately covered with plastic bags for 48 hrs and were maintained. Per cent incidence and severity was recorded before spraying. After that each of five plants were sprayed with ca. 20±5 ml of one of the treatments. After 14 days, per cent disease incidence and severity was recorded (Graham and Leite, 2004).

Second spraying was done at 15th day and observations were recorded after 14 days. The experiment was carried out in completely randomized design with 10 treatments and 3 replicates with two spraying. One of the treatments including five seedlings inoculated with bacterial suspension as positive control. Five seedlings were sprayed only with sterile distilled water as negative control (Samavi *et al.*, 2009).

Experimental details

Design : CRD (Completely Randomized Design)
Replication : Three
Treatments : Twelve
Variety : Kesar

Treatment details:

Sr. No.	Treatments	Concentration
T_1	*Psuedomonas fluorescens*	2 x 10^8 CFU/ml
T_2	*Bacillus subtilis*	2 x 10^8 CFU/ml
T_3	*Psuedomonas striata*	2 x 10^8 CFU/ml
T_4	Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w (Streptocycline)	RD @ 125 %
T_5	Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (Plantomycin)	RD @ 125 %
T_6	Kasugamycin 5%+ Copper oxycloride 45% WP(Conika)	RD @ 125 %
T_7	Copper oxychloride 50% WP	RD @ 125 %
T_8	Mancozeb 75% WP	RD @ 125 %
T_9	Cymoxanil 8% + Mancozeb 64% WP	RD @ 125 %
T_{10}	Cinamon oil	2000ppm
T_{11}	Neem oil	2000ppm
T_{12}	Control	--

3.8. STATISTICAL ANALYSIS

The data obtained in all the experiments (*in vitro and* pot culture) were statistically analyzed (Panse and Sukhatme, 1978) using VNMAU-STAT statistical programme, at Central Computer Laboratory, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

CHAPTER-IV

RESULTS AND DISCUSSION

CHAPTER-IV

RESULTS AND DISCUSSION

Bacterial leaf spot disease of mango caused by *Xanthomonas campestris* pv. *mangiferaeindicae* is one of the most destructive disease of mango infecting considerable quantitative and qualitative losses. Mostly the disease occurred on leaves, fruits and stems. Considering the economic importance of the fruit crop as well as disease, present investigations were undertaken during 2018-19, 2019-20 and 2020-21 on the aspects *viz.,* collection of diseased samples from different agro-climatic zones of Marathwada region, symptomatology, isolation, pathogenecity, morphocultural, biochemical and molecular characterization, epidemiological studies, *in vitro* efficacy of antibiotics, fungicides, bioagents, essential oils and integrated disease management of bacterial leaf spot of mango. The results obtained on all these aspects are being interpreted and discussed under following sub-heads.

4.1 Collection of mango bacterial leaf spot diseased specimens from Marathwada region

Collection of mango bacterial leaf spot diseased samples were carried out during 2017-18 from three agro-climatic zones of Marathwada region. During collection record of was maintained *viz.,* plant age, variety, disease intensity of bacterial leaf spot on mango from different locations in different tahsils of Marathwada region.

Table 4.1: Collection of mango bacterial leaf spot from different Tahsil of Marathwada region during 2018 - 19

Sr. No.	Districts	Tehsils	Location	Age of Plant (Yr.)	Variety	Plant part collected	Intensity
Scarcity zone							
1.	**Aurangabad**	Gangapur	Gangapur	8	Dasheri	Leaves	23.50
2.	**Beed**	Parli	Anandwadi	8	Local	Leaves, Fruits	45.30
		Georai	Belgaon	9	Local	Leaves	42.22
3.	**Osmanabad**	Bhoom	Ashta	7	Kesar	Leaves, Stem	24.11
Average				8			33.78

Assured rainfall zone							
1.	**Aurangabad**	Aurangabad	Pachod	7	Kesar	Leaves, Stem, Fruits	25.12
		Paithan	Paithan	9	Kesar	Leaves	22.12
2.	**Jalna**	Ambad	Antarweli	8	Kesar	Leaves, Stem	20.12
		Jalna	Jalna	8	Dasheri	Leaves, Fruits	21.12
		Mantha	Mangrul	9	Local	Leaves	21.56
3.	**Beed**	Ambajogai	Girwali	7	Local	Leaves	42.10
		Beed	Ashti	7	Kesar	Leaves	43.25
		Majalgaon	Majalgaon	6	Local	Leaves, Fruits	42.12
		Keij	Keij	8	Local	Leaves	41.33
4.	**Osmanabad**	Tuljapur	Dhangarwadi	6	Local	Leaves, Stem	25.12
		Kalamb	Kalamb	7	Dasheri	Leaves	22.13
		Osmanabad	Alni	8	Kesar	Leaves	22.11
5.	**Latur**	Ahmedpur	Dagadwadi	9	Local	Leaves	21.96
		Udgir	Hipparga	9	Neelam	Leaves, Fruits	26.12
		Latur	Latur	7	Kesar	Leaves	23.46
		Nilanga	Madansuri	5	Kesar	Leaves	24.87
6.	**Parbhani**	Manwath	Kolha	9	Local	Leaves	29.12
		Jintur	Bori	9	Kesar	Leaves, Fruits	26.13
		Parbhani	Zari	9	Neelam	Leaves	22.47
		Pathri	Renapur	8	Kesar	Leaves	22.96
		Gangakhed	Makhni	7	Dasheri	Leaves, Fruits	23.12
7.	**Nanded**	Kandhar	Kandhar	8	Local	Leaves, Fruits	20.11
		Deglur	Markhel	8	Neelam	Leaves	24.23
		Loha	Loha	7	Dasheri	Leaves	29.12
8.	**Hingoli**	Sengaon	Yeldari	7	Local	Leaves, Fruits	31.12
Average				7.68			26.92
Moderate rainfall zone							
1.	**Nanded**	Mudkhed	Mudkhed	5	Kesar	Leaves	33.12
		Nanded	Vishnupuri	9	Kesar	Leaves, Fruits	36.45
		Himayatnagar	Himayatnagar	7	Local	Leaves	33.98
2.	**Hingoli**	Aundha	Aundha	8	Dasheri	Leaves	32.69
		Hingoli	Hingoli	8	Local	Leaves, Fruits	35.98
		Basmat	Basmat	6	Local	Leaves	34.78
Average				7.2			34.50

Plate 4.1. Collection of mango bacterial leaf spot, during 2018-19

Results (Table 4.1) revealed that mango orchards / plants in all agro-climatic zones are prone to bacterial leaf spot of mango exhibiting different per cent disease intensity and it ranged from 20.11 to 45.30 per cent amongst three agro-climatic zones of Marathwada.

From scarcity zone (SC), total four disease samples of mango bacterial leaf spot were collected where it ranged from 23.50 to 45.30 per cent. However, maximum intensity was recorded in Anandwadi (45.30 %) on local variety, followed by Sirsala (42.22 %) on local variety, Ashta (24.11 %) on variety kesar while minimum per cent intensity was recorded at location Gangapur on variety Dasheri (23.50 %).

Total twenty five leaf samples of mango bacterial leaf spot were collected from Assured rainfall zone (AR) where disease intensity ranged from 20.11 to 43.25 per cent. However, maximum per cent disease intensity was recorded in Ashti (43.25 %) on cultivar Kesar, followed by Majalgaon (42.12 %) on local variety, Girwali (42.10 %) on local variety, Keij (41.33 %) on local variety, Kolha (29.12 %) on local variety and Loha (29.02 %) on Dasheri; Whereas, minimum per cent intensity was recorded from Kandhar on local variety (20.11 %).

From moderate rainfall zone, total six samples of mango bacterial leaf spot were collected. The disease intensity ranged from 32.69 to 36.45 per cent. However, maximum bacterial leaf spot intensity was recorded in Vishnupuri on Kesar variety of mango (36.45 %) followed by Hingoli (35.98 %) on local variety, Basmat (34.78 %) on local variety, Himayatnagar (33.98 %) on local variety and Mudkhed (33.12 %) on Kesar; while minimum per cent intensity was recorded in Aundha (32.69 %) on Dasheri variety.

Table 4.2: Collection of mango bacterial leaf spot from different Agro-climatic zones of Marathwada region during 2018-19

Sr. No.	Agro-climatic Zone	No. of locations	Average Intensity (%)
1	Scarcity zone	04	33.78
2	Assured rainfall zone	25	26.92
3	Moderate rainfall zone	06	34.50
	Overall Average / Total	**35**	**31.73**

Data presented in Table 4.2 & Fig. 4.1, showed that average per cent disease intensity of mango bacterial leaf spot among three agro-climatic zones ranged from 26.92 to 34.50 per cent. However, maximum average per cent disease intensity was recorded in Moderate rainfall zone (34.50%) followed by Scarcity zone (33.78%); whereas, minimum average per cent disease intensity was recorded in Assured rainfall zone (26.92%).

Table 4.3: Collection of mango bacterial leaf spot from different District of Marathwada region during 2018-19

Sr. No.	District	No. of locations	Av. Intensity (%)
1	Aurangabad	03	23.58
2	Beed	06	42.72
3	Hingoli	04	33.64
4	Jalna	03	20.93
5	Latur	04	24.10
6	Nanded	06	29.50
7	Osmanabad	04	23.36
8	Parbhani	05	24.76
	Overall Average / Total	**35**	**31.73**

Data presented in Table 4.3 & Fig. 4.2, revealed that maximum average intensity of the disease was found in Beed district (42.72 %) followed by Hingoli district (33.64 %) during 2017-18. Whereas, in Jalna district minimum average disease intensity *i.e*. 20.93 % was recorded.

4.2 Symptomotology

Symptoms were observed on leaves, fruits, branches and twigs. Initially small, water soaked lesions delineated by veins were noticed on lower surface of the leaves followed by appearance of small brown to dark brown coloured spots on the upper surface with circular to irregular shape with chlorotic halo. As the disease progressed, these spots also grew, increased in size, coalesced and extended upto midrib in a week's time covering the major portion of leaf lamina. Severely infected leaves turned yellow, became chlorotic and finally dropped down. On developing green fruits, symptoms were noticed as small pin head sized, black lesions

Plate 4.2. Symptoms on leaves

Plate 4.3. Symptoms on fruits

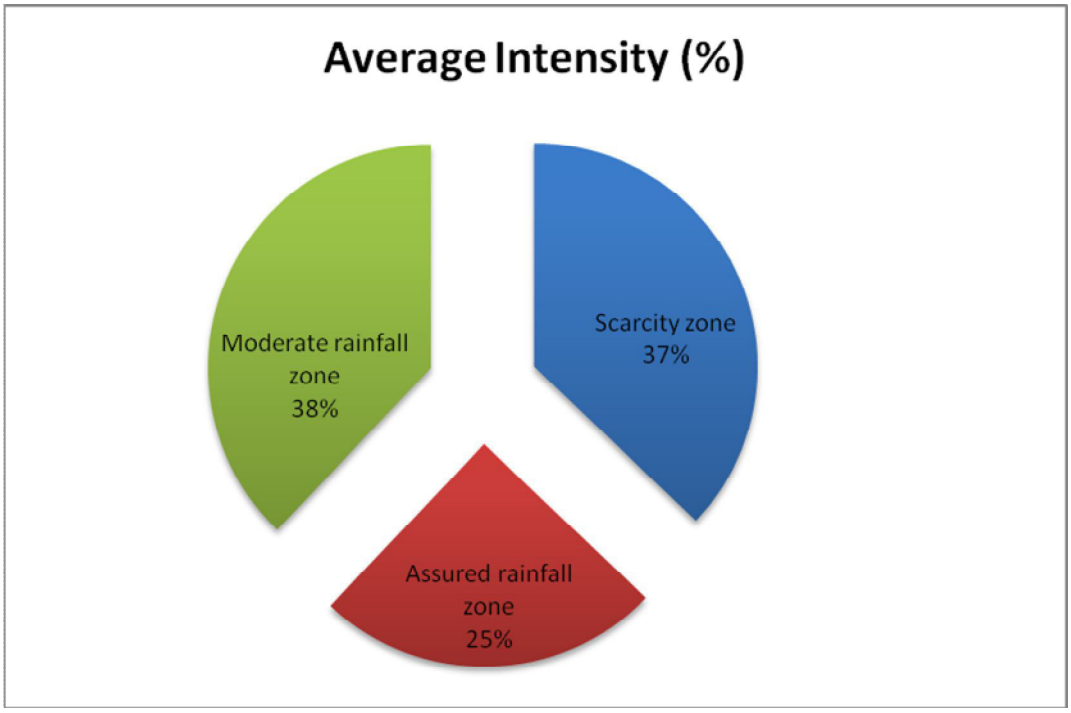


Fig. 4.1. Agro-climatic zone wise average disease intensity

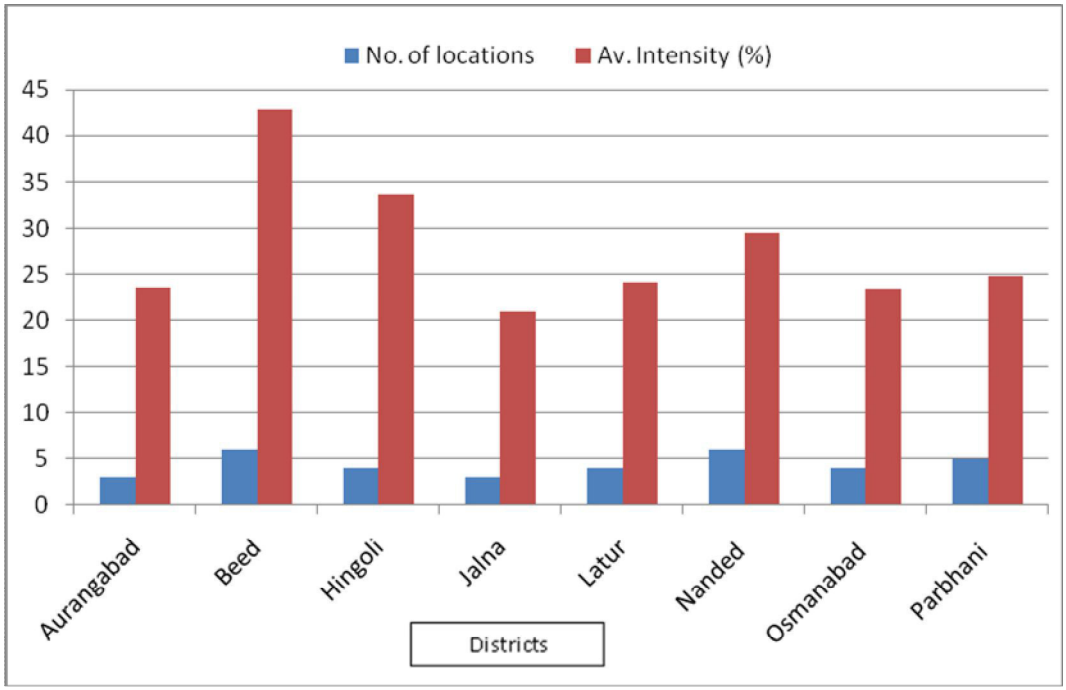


Fig. 4.2. District wise average disease intensity

with diffused water soaked margin, which later developed into black coloured, medium to big sized erumpent cankerous spots with sometimes showing characteristics tear eye symptoms with bacterial shining exude. Symptoms on twigs and branches were noticed as longitudinal cracks which later developed into cankerous lesions. The results were shown in Plate 4.2 & 4.3.

During the present studies, symptoms observed on mango plants infected naturally with bacterial leaf spot (*X. campestris pv. mangiferaeindicae*) were similar to those symptoms induced by *X. campestris pv. mangiferaeindicae* in many mango orchards reported earlier by several workers. Such as from Maharashtra (Patel *et al.,* 1948), and other parts of the country (Shekhawat and Patel, 1978), Uttar Pradesh (Mishra and Om Prakash, 1992), Karnataka (Thirumalesh *et al.,* 2012) and West Bengal (Khatua *et al.,* 2013). Gangnevin and Provost (2001) reported similar symptoms on leaves, twigs, branches and fruits in South Africa. Leaf and fruit symptoms were most common but twig and branch canker occurred when infection was severe. Leaf symptoms began as small, water soaked spot delineated by veins, becoming raised, black, some with chlorotic halo. Fruit symptoms appeared as small water soaked spot on lenticels and they later become star shaped, and exuded an infectious gum. Recently similar symptoms of mango bacterial leaf spot were reported by Irfan *et al.,* (2017). They observed lesion development on leaves, twigs and fruits. Leaf lesions were black, raised and tend to be angular in shape as they were confined by the large veins frequently surrounded by yellow margins. Bacterial lesions do not expand with fruit ripening. Twig and stem lesions were black and cracked. Spots on fruits were black often with star shaped cracks and water soaked margins. Bandi (2019) observed the mango bacterial leaf spot symptoms as, initially small water soaked lesions delineated by veins on the lower surface of leaves followed by appearance of small brown to black coloured spots on the upper surface with round to irregular shape with chlorotic halo. In initial stages symptoms on fruit were noticed as small, pin head sized, black lesions with diffused water soaked margins, which later developed into cankerous growth.

4.3 Isolation of the bacterium

The bacterium (*X. campestris pv. mangiferaeindicae*) was successfully isolated by bacterial ooze obtained from the leaves of naturally bacterial leaf spot

affected mango plants, on nutrient agar (NA). The colonies of the bacterium were developed within 48 hrs. of incubation. Then these bacterial colonies were transferred on fresh nutrient agar plates by streak plate method. The pathogen was purified through frequent sub culturing and purified growth or culture was maintained on fresh nutrient agar slants and stored at 28 ± 2 ^{0}C temperature for further studies.

Applying same procedure, 35 isolates of *X. campestris* pv. *mangiferaeindicae* were isolated (Table 4.4) and assigned them the nomenclature Xcm1, Xcm2, Xcm3, Xcm4, Xcm5, Xcm6, Xcm7 upto Xcm35. The culture of these isolates were purified by streak plate method and transferred on nutrient agar slant and stored at 28 ± 2^{0}C temperature and maintained separately for further *in vitro* studies.

The results of present study on *X. campestris* pv. *mangiferaeindicae* correlates with reports of Moffett *et al.,* (1977), Manicom and Wallis (1984), Dayakar and Gnanamanickam (1996), Najafipour *et al.,* (2014), Ofoe *et al.,* (2016). Strains of bacteria causing bacterial leaf spot of mango isolated using nutrient agar medium (Manicom and Wallis, 1984). Rashid *et al.,* (2016) isolated bacterial leaf spot pathogen from mango leaves by dilution plate technique. Modified nutrient glucose medium was used for bacterial colony isolation and recovered colonies were purified by streaking method. Bandi (2019) isolated *Xanthomonas campestris* pv. *mangiferaeindicae* from the infected leaf and fruit parts of mango plant by following the streak plate technique using nutrient agar medium. The colonies appeared after four two six days interval of time after the incubation. Culture of each isolate was purified by streaking suspected single colony on nutrient agar medium and maintained on slants.

4.4 Identification of pathogen

Based on symptomatology of naturally / artificially inoculated bacterial leaf spot diseased mango plants, morphocultural characteristics, Gram staining, biochemical tests, pathogenecity test, microscopic observations the test pathogen was identified as *X. campestris* pv. *mangiferaeindicae,* the cause of bacterial leaf spot of mango described by Ah-You *et al.* (2007) and Thirumalesh (2012).

4.5 Pathogenecity

The pathogenecity of *X. campestris* pv. *mangiferaeindicae* was established on leaves of mango cv. Kesar. The inoculums of bacterial pathogen was prepared and pathogenecity was proved by applying detached leaf assay in controlled conditions.

The leaves treated with sterile distilled water served as control. Both inoculated and controlled leaves were kept in BOD incubator for development of typical symptoms of disease which were developed within 9 to 14 days after inoculation on leaves. Result revealed that (Plate 4.4 (A & B) and Fig. 4.3) all the test isolates did not induce the symptoms up to 7^{th} day of incubation. From 8^{th} day onwards symptoms were noticed in some isolates *viz.,* Xcm-2, Xcm-6, Xcm-11, Xcm-14, Xcm-16, Xcm-22, Xcm-26, Xcm-28, Xcm-30, Xcm-31 & Xcm-35. All the isolates showed same type of symptoms but variations were observed in shape of the spots, incubation period and number of spots and size of the spots. The symptoms were observed as initially small, water soaked lesions delineated by veins on the lower surface of the leaves followed by appearance of small brown to black coloured spots on the upper surface. The spots were circular to irregular shape with yellow halo. As the disease progressed, these spots also grew, increased in size coalesced and extended upto midrib in a week's time covering the major portion. Average incubation period (days) varied from 9.66 (Xcm4 and Xcm12) to 14.13 (Xcm20) on leaves of Kesar cultivar of mango. Maximum incubation period (days) was in isolate Xcm20 (14.13) followed by Xcm9 (14.00), Xcm21 (13.99) and Xcm6, Xcm24 and Xcm30 (13.66 days). While minimum average incubation period was in Xcm5 and Xcm12 (9.66 days) followed by Xcm2 and Xcm35 (10.33 days).

Table 4.4: Pathogenic variability of the isolates *Xanthomonas campestris pv. mangiferaeindicae*

S. N.	Isolates	Symptoms observed after incubation period (Days)*								No. of days required for initiation of symptoms	Av. No. of spots*	Av. size of spot (mm)*
		Upto 7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day			
1	Xcm1	-	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Brown to dark brown, irregular spots	Yellow halo around the spots		11.33	2.33	2.33
2	**Xcm2**	No symptoms	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Brown to dark brown, irregular spots	Yellow halo around the spots				10.33	3.00	1.33
3	Xcm3	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Brown to dark brown, irregular spots	Yellow halo around the spots			12.00	4.00	2
4	Xcm4	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Brown to dark brown, circular to irregular spots	Yellow halo around the spots			13.13	3.33	2.66
5	**Xcm5**	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, irregular spots	Yellow halo around the spots			9.66	3.00	1.66
6	Xcm6	-	Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Brown to dark brown, irregular spots	Yellow halo around the spots				13.66	2.33	2.33
7	**Xcm7**	-	-	-	Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Light to dark brown, irregular spots	Yellow halo around the spots		10.99	4.00	2.33
8	Xcm8	-	-	-	Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Brown to dark brown, irregular spots	Yellow halo around the spots		11.33	5.33	2.00

9	Xcm9	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Brown to dark brown, irregular spots	Yellow halo around the spots			14.00	3.66	2.33
10	Xcm10	-	-		Water soaked lesions delineated by veins, on lower	Water soaked lesions on upper surface of leaves	light to dark brown, circular to irregular	Yellow halo around the spots		12.66	4.00	1.99
11	Xcm11	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots				11.66	2.00	1.66
12	Xcm12	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Yellow halo around the spots				9.66	3.00	2.33
13	Xcm13	-	-			Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular spots	Yellow halo around the spots	11.33	3.99	2.00
14	Xcm14	-	Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots				12.66	5.00	2.66
15	**Xcm15**	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots			10.33	4.00	1.99
16	Xcm16	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots		Yellow halo around the spots	12.13	5.33	1.66
17	Xcm17	-	-			Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular	Yellow halo around the spots	13.13	3.66	2.33

18	Xcm18	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Yellow halo around the spots				10.66	4.00	2.00
19	**Xcm19**	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots			11.13	3.66	1.99
20	Xcm20	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots			13.99	3.00	1.66
21	Xcm21	-	-	-	-	-	Water soaked lesions delineated by	Dark brown, circular to irregular spots	Yellow halo around	12.66	4.33	1.66
22	Xcm22	-	Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Dark brown, circular to irregular spots	Yellow halo around the spots				13.33	2.66	2.33
23	**Xcm23**	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Dark brown, circular to irregular spots	Yellow halo around the spots			10.66	4.33	2.66
24	Xcm24	-	-		Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Dark brown, circular to irregular spots	Yellow halo around the spots		13.66	3.00	2.13
25	Xcm25	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots			11.13	5.00	2.99
26	Xcm26	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, irregular spots	Yellow halo around the spots				10.66	4.33	1.66

27	Xcm27	-	-		Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Light to dark brown, irregular spots	Yellow halo around the spots		11.13	4.13	2.33
28	Xcm28	-	Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots				11.33	4.00	1.33
29	Xcm29	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots			13.66	3.99	2.66
30	**Xcm30**	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots				10.33	5.32	2.66
31	Xcm31	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots				12.66	5.00	2.00
32	Xcm32	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Dark brown, circular to irregular spots	Yellow halo around the spots			12.33	4.00	1.99
33	**Xcm33**	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, circular to irregular spots	Yellow halo around the spots			11.33	3.00	2.66
34	Xcm34	-	-	Water soaked lesions delineated by veins	Water soaked lesions on upper surface of leaves	Light to dark brown, irregular spots	Yellow halo around the spots			11.33	5.00	2.33
35	Xcm35	-	Water soaked lesions delineated by veins, on lower leaves	Water soaked lesions on upper surface of leaves	Dark brown, circular to irregular spots	Yellow halo around the spots				10.33	3.66	1.99

Average number of spots varied from 2.00 (Xcm11) to 5.33 (Xcm7, Xcm15, Xcm30,). However, maximum number of spots was in three isolates *viz.,* Xcm7, Xcm15, Xcm30 exhibited same average number of spots *i.e* 5.33, followed by four isolates *viz.* Xcm14, Xcm23, Xcm31, Xcm33 exhibited same average number of spots *i.e* 5.00, while minimum average number of spots was in Xcm11 (2.00) followed by two isolates Xcm1 and Xcm6 (2.33). Average size of spots (mm) was varied from 1.33mm (Xcm1 and Xcm28) to 2.99mm (Xcm2). However, maximum average size of spot was observed in Xcm23 (2.99 mm), followed by five isolates viz., Xcm5, Xcm15, Xcm25, Xcm30 and Xcm33 exhibited same average size of spot i.e 2.66 mm, followed by nine isolates Xcm2, Xcm6, Xcm7,Xcm9, Xcm12, Xcm17, Xcm22, Xcm27 and Xcm34 exhibited same average size of spot i.e 2.33 mm.While minimum average size of spots was in two isolates Xcm1 and Xcm28 (1.33 mm)

Observations were made regularly for the appearance and development of symptoms in artificially inoculated leaves, re-isolation of the pathogen was done and culture so obtained was compared with the original culture for the confirmation of Koch's postulate. Thus, all isolates confirmed to cause bacterial leaf spot disease on inoculated leaves of mango. On the basis of pathogenecity, one isolate from each district of Marathwada region as, Xcm2, Xcm5, Xcm7, Xcm15, Xcm19, Xcm23, Xcm30, and Xcm33 was selected for further studies. (Plate - 4.5)

Table 4.5: Redesignation of isolates for further studies

Sr. No.	Isolate code		Location	Agro-climatic zone
	Old	New		
1	Xcm2	Xcm1	Anandwadi	SC
2	Xcm5	Xcm2	Pachod	AR
3	Xcm7	Xcm3	Antarweli	AR
4	Xcm15	Xcm4	Kalamb	AR
5	Xcm19	Xcm5	Latur	AR
6	Xcm23	Xcm6	Zari	AR
7	Xcm30	Xcm7	Mudkhed	MR
8	Xcm33	Xcm8	Aundha	MR

Similarly, pathogenecity of *X. campestris* pv. *mangiferaeindicae,* by different methods causing bacterial leaf spot of mango was proved successfully. The

Plate 4.4(A). Pathogenecity of 35 isolates collected from Marathwada region

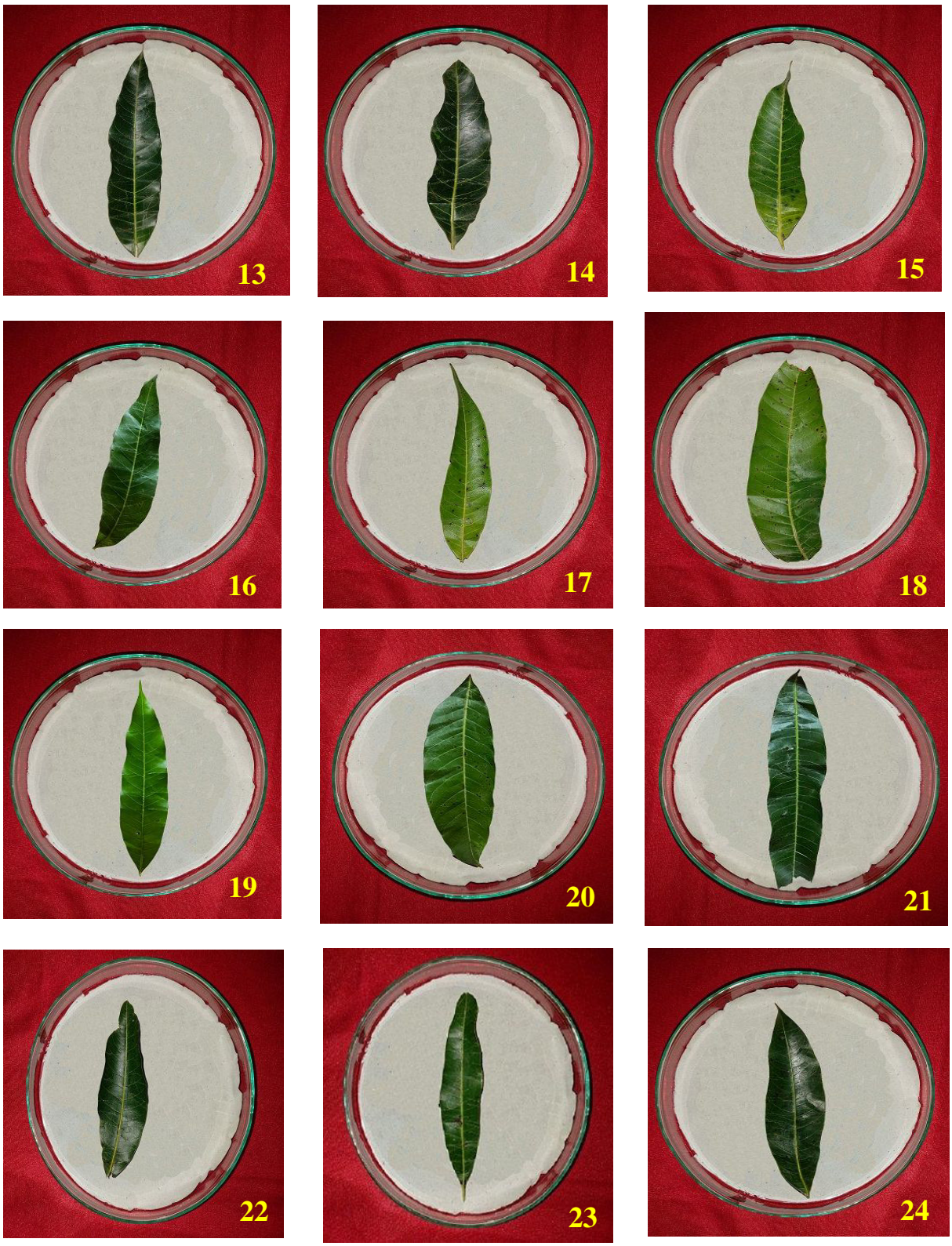


Plate 4.4(B). Pathogenecity of 35 isolates collected from Marathwada region

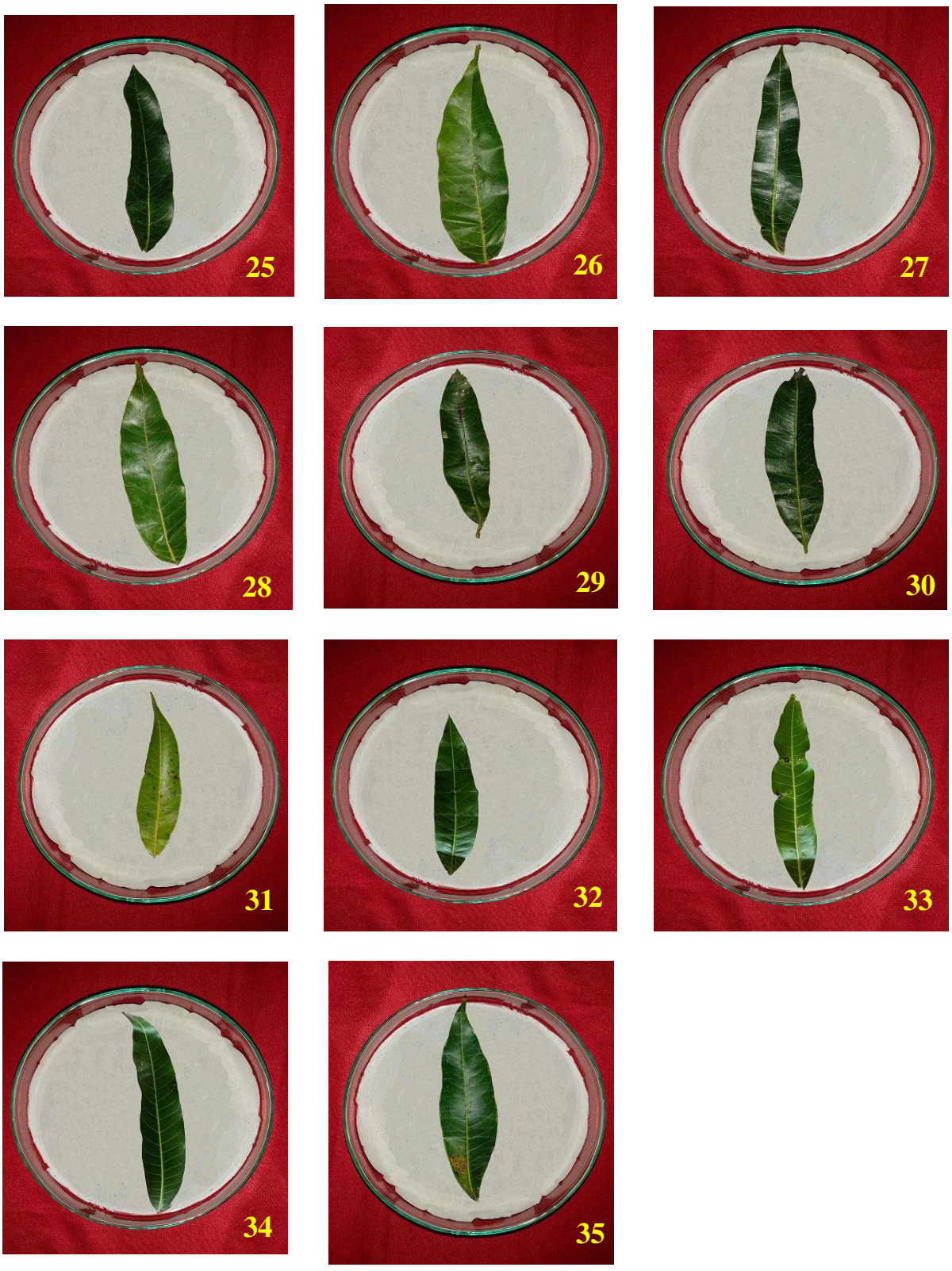
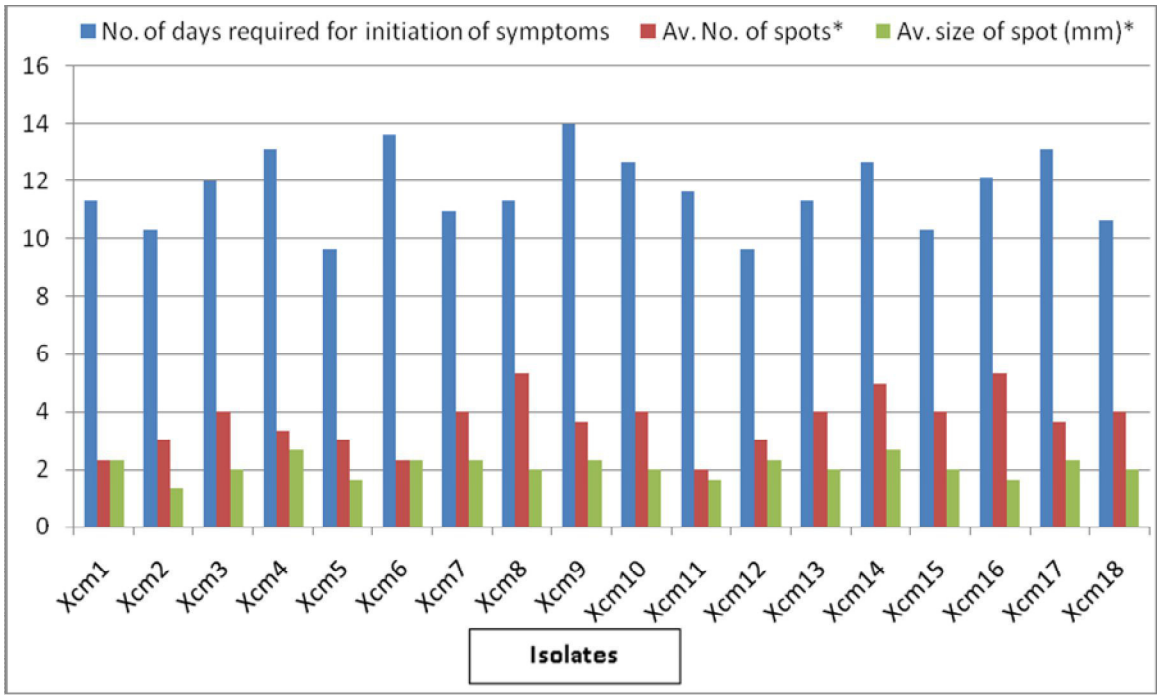


Plate 4.4(C). Pathogenecity of 35 isolates collected from Marathwada region

Isolates	Locations
Xcm-1	Anandwadi
Xcm-2	Pachod
Xcm-3	Antarweli
Xcm-4	Kalamb
Xcm-5	Latur
Xcm-6	Zari
Xcm-7	Mudkhed
Xcm-8	Aundha

Plate 4.5. Pure culture of *X. campestris* pv. *mangiferaeindicae*



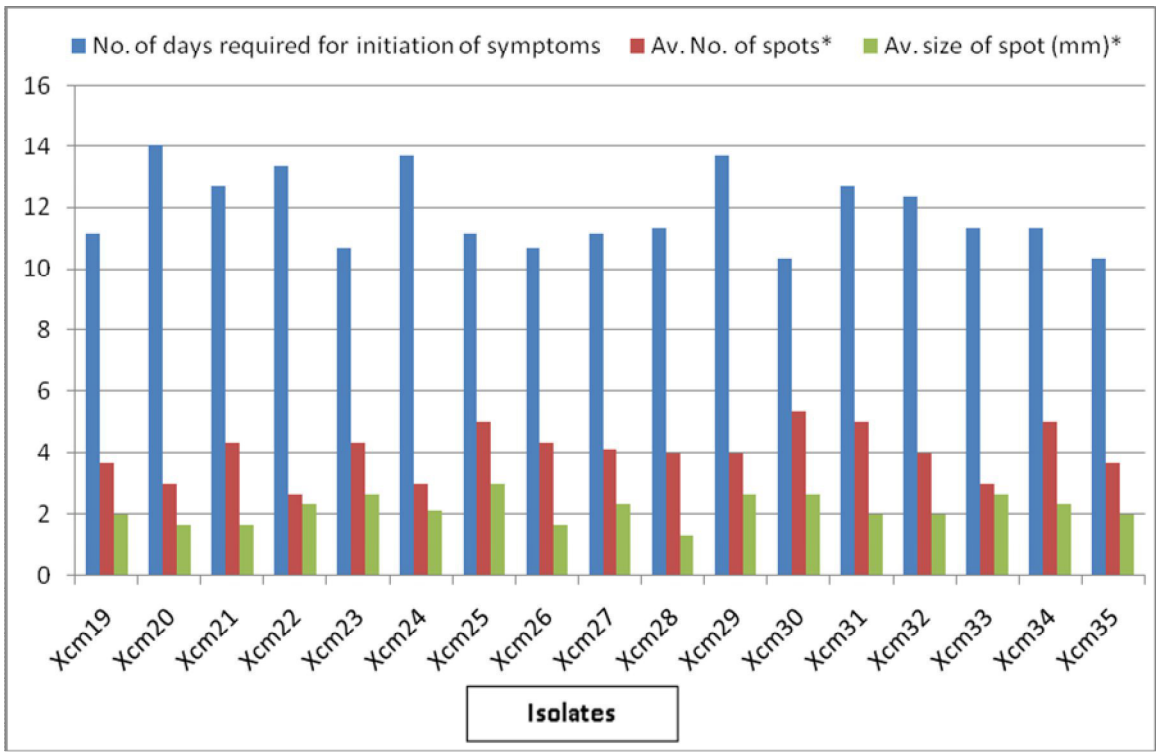


Fig. 4.3. Pathogenic variability among the all 35 *Xcm* isolates collected from Marathwada region

pathogenic association of *Xanthomonas* spp. was established in various mango varieties, fruit and vegetables etc. earlier by several workers. (Manicon and Wallis, 1984; Manjula, 2002; Thirumalesh *et al.,* 2012; Pawar *et al.,* 2014; Snahuja, 2016; Zombre *et al.,* 2016; Bhure *et al.,* 2019). Bandi (2019) proved the pathogenecity of *X. campestris* pv. *mangiferaeindicae* isolates and also pathogenic variability among isolates was studied regarding lesion size measured 12 and 15 days after inoculation and appearance of symptoms.

4.6 Studies on variability among the isolates of *X. campestris* pv. *mangiferaeindicae* isolates

4.6.1 Symptomatic variability of virulent pathogen in the field

During the collection of samples bacterial leaf spot symptoms expressed variability in respect of their lesion shape, size and colour etc. (Table 4.6 and Plate 4.4 (A & B))

Table 4.6: Symptomatic variability among the bacterial leaf spot specimens collected from different agro climatic zones of Marathwada region

Isolate name	Agro climatic zone	Location	Symptoms		
			Lesion shape	Lesion size (mm)	Lesion colour
Xcm1	SC	Anandwadi	Circular to irregular	2	Dark brown with yellow halo
Xcm2	AR	Pachod	Circular to irregular	2.5	Brown with yellow halo
Xcm3	AR	Antarweli	Circular to irregular	2	Dark brown
Xcm4	AR	Kalamb	Circular to irregular	2	Light brown with yellow halo
Xcm5	AR	Latur	Circular to irregular	1	Brown with yellow halo
Xcm6	AR	Zari	Circular to irregular	1	Black
Xcm7	MR	Mudkhed	Circular to irregular	3	Dark brown with yellow halo
Xcm8	MR	Aundha	Circular to irregular	2	Brown with yellow halo

Results (Table 4.6, Plate 4.4 (A & B)) revealed that lesion shape were circular to irregular on mango leaves, grown in the all agro-climatic zone of the Marathwada region. The size of the leaf lesion was also varied, ranged from 1 mm to

3 mm in dia., maximum lesion size was found in Mudkhed (Xcm7) isolate (3mm) from moderate rainfall zone followed by Antarweli (Xcm2), Pachod (Xcm1), Anandwadi (Xcm3), Kalamb (Xcm4) and Aundha *i.e* 2 mm from three agro-climatic zones of marathwada region. However, minimum lesion size was found in isolates Xcm5 and Xcm6 collected from latur and zari respectively Assured rainfall zone *i.e* 1 mm.

Colour of lesion on mango leaves was mostly brown with yellow halo in agro-climatic zones *viz.,* SC, AR, MR and dark brown with yellow halo in agro-climatic zones *viz.,* AR and MR.

4.6.2 Studies on different inoculation techniques for Xcm on leaves

The pathogenecity test was performed with pure culture of the bacterial pathogen isolated from diseased sample affected with *X. campestris* pv. *mangiferaeindicae* by three different methods for confirming the pathogenecity of the causal bacterium. The data on standardization of inoculation methods is presented in Table 4.7.

Table 4.7: Standardization of Inoculation Methods for Xcm

Inoculation method	No. of days required for initiation of symptoms	No. of spots observed after inoculation	Symptom appearance	CFU/ml observed after re-isolation
Pin-prick method	12.33	2.33	Water soaked, circular to irregular, dark brown spots	10×10^6
Infiltration method	9.33	3	Water soaked, circular to irregular, dark brown spots	12×10^6
Spray Inoculation method	14	2	Water soaked, circular to irregular, dark brown spots	8×10^6

The data revealed that amongst three different methods, infiltration methods was found most suitable for development of symptoms which had taken average 9.33 days for symptom development. The method was followed by pin prick method (12.33 days) and spray inoculation (14 days). Maximum number of lesions *i.e* 3 lesion on leaves of mango was observed in the plants inoculated with infiltration

Plate 4.6(A). Symptomatic variability among the bacterial leaf spot specimens

Plate 4.6(B). Symptomatic variability among the bacterial leaf spot specimens

method followed by pin prick method and spray inoculation method. After re-isolation, maximum number of colony forming units of Xcm were observed in case of infiltration method followed by pin prick method and spray inoculation method.

Amongst all three methods, adopted for inoculation on the pathogen, infiltration method was found very good for establishment of pathogen inside the host. The re-isolation attempted from artificially infected diseased plant tissues on Nutrient Agar consistently yielded *X. campestris* pv. *mangiferaeindicae,* thus fulfilling Koch's postulates and association of *X. campestris* pv. *mangiferaeindicae* with mango was confirmed.

Similarly, out of three methods attempted to prove the pathogenecity of *X. campestris* pv. *mangiferaeindicae,* infiltration method was effective in proving the pathogenic association of *Xanthomonas* spp. in various mango varieties, fruit and vegetables etc. The findings of earlier workers were also in agreement with the present study. (Manicon and Wallis, 1984; Manjula, 2002; Thirumalesh *et al.,* 2012; Pawar *et al.,* 2014; Snahuja, 2016; Zombre *et al.,* 2016; Bhure *et al.,* 2019).

4.6.3. Pathogenic variability

The pathogenic variability among the eight isolates of *X. campestris* pv. *mangiferaeindicae viz.,* Xcm1, Xcm2, Xcm3, Xcm4, Xcm5, Xcm6, Xcm7 and Xcm8 collected and isolated from different agro-climatic zones of Marathwada region was detected by two separate methods *viz.,* attached leaf assay and detached leaf assay.

4.6.3.1. Attached leaf assay

The pathogenic variability among the eight isolates of *X. campestris* pv. *mangiferaeindicae* were studied by attached leaf technique under screen house condition and susceptible variety of mango *i.e* Kesar was used.

The results (Table 4.8, Plate 4.7 (A & B) and Fig. 4.4) revealed that, all eight isolates of *X. campestris* pv. *mangiferaeindicae* were pathogenic and caused bacterial leaf spot in mango (Kesar). Average incubation period varied from 12 (Xcm2) to 14 (Xcm7) days on mango among eight isolates. Maximum incubation

period was recorded in isolate Xcm7 from zone MR (14). However, in all seven isolates, the period varied non significantly from 12.00 to 13.33 days.

The number of spots on leaves also varied from 2.66 to 4 in numbers. Significantly more numbers of spot found in Xcm8 isolate (4.00) from agro-climatic zone MR, followed by lesser number of spots in rest of the isolates varied non significantly from 2.66 to 3.76.

Table 4.8: Pathogenic variability among the *Xcm* isolates (attached leaf assay)

Sr. No.	Isolates	Agro climatic Zone	Av. Incubation period (Days)*	Av. No. of spots*	Av. Size of spot (mm)*
1	Xcm1	SC	12.66	3	2
2	Xcm2	AR	12	2.66	1.66
3	Xcm3	AR	12.66	2.76	1.76
4	Xcm4	AR	13.33	3	1.83
5	Xcm5	AR	12.33	3.66	2.26
6	Xcm6	AR	13	3.33	1.83
7	Xcm7	MR	14	3.76	2.5
8	Xcm8	MR	13.33	4	2.16
S.E. ±			**0.47**	**0.37**	**0.28**
C.D. (P= 0.01)			**1.38**	**1.09**	**0.84**

*Mean of three replications

Size of leaf spot also varied significantly ranging from 1.66 to 2.5 mm in diameter. There was no significant difference in spot diameter in any of the isolates.

On the basis of results it has been inferred that the mango bacterial incubation period, number of spot and size were varied on Kesar cultivar due to isolates collected from different regions of Marathwada falling under various agro-climatic conditions it may be due different genetic makeup of isolates.

Similar results were also reported earlier by many workers. Tsuchiya *et al.,* (2003) tested pathogenecity using two year old plants. The tests were done on whole plants using an automizer where young green twigs and mature green leaves were wounded with needles and sprayed with inoculums prepared by standardizing cell suspensions about 10^8 cfu/ml. The plants were covered with polyethylene bags and kept in greenhouse at 20^0 - 28 ^{0}C under natural light. Bhure *et al.,* (2019) studied

Plate 4.7(A). Pathogenicity test (Attached leaf assay)

Plate 4.7(B). Pathogenicity test (Attached leaf assay)

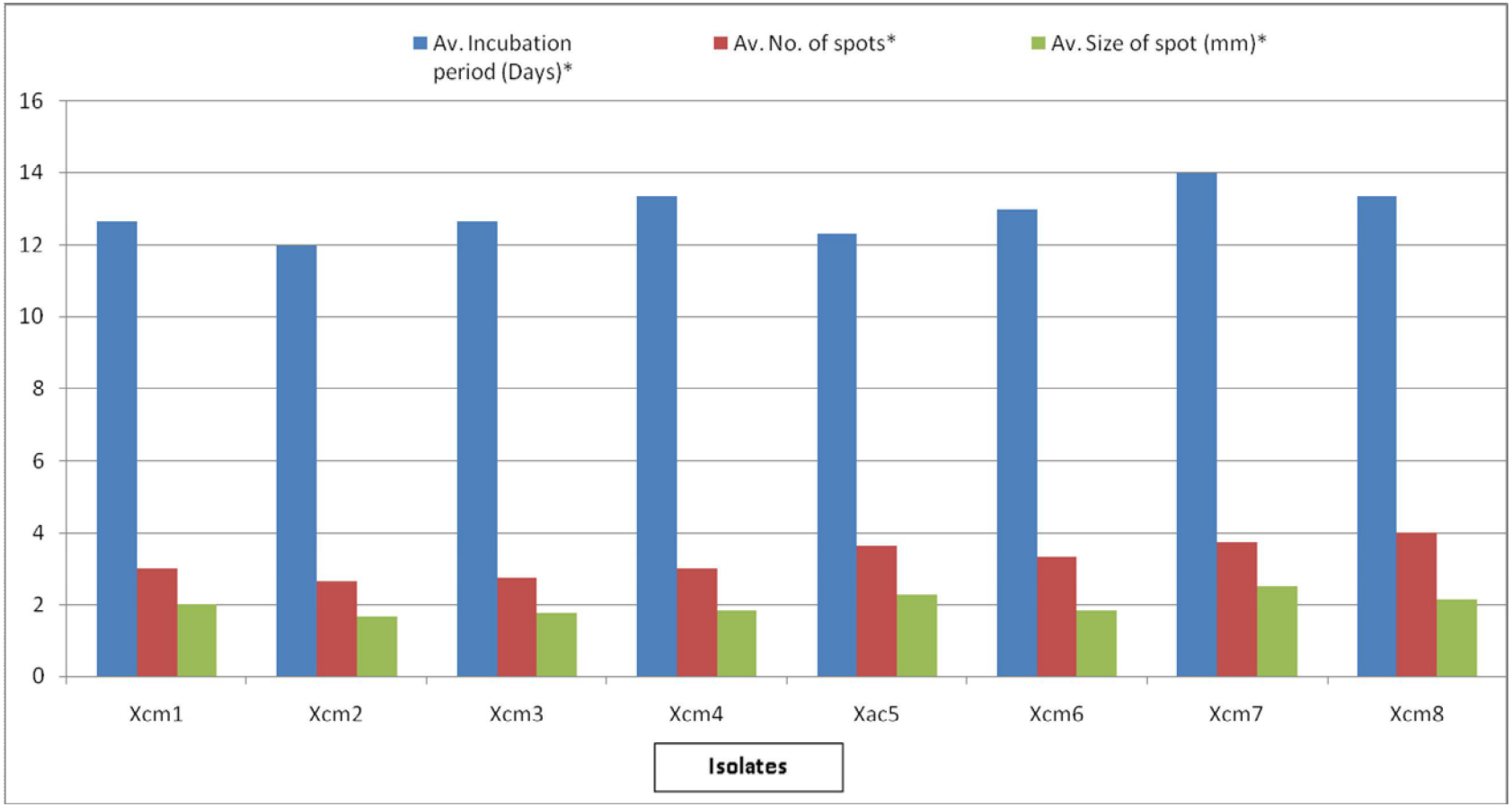


Fig. 4.4. Pathogenic variability among the *Xcm* isolates (attached leaf method)

in vivo pathogenic variability by Pot culture (seedling inoculation) technique among ten isolates of *X. axonopodis* pv. *citri* which were isolated from different regions of Vidarbha. Results showed that all isolates exhibited symptoms between ranges of 15 (Xac 10) to 25 DAI *in vivo* and conformed that isolate Xac-10 showed highly pathogenic to initiate water soaked lesion and fully developed symptoms after 15 days under *in vivo* condition. While isolate Xac-10 (Akola) gave 3 mm water soaked lesions surrounded by yellow halo zone; whereas Xac-2, Xac-3, Xac-5, Xac-6 and Xac-7 were found weak canker lesions and Xac-1, Xac-4, Xac-8 and Xac-9 were found moderate canker lesions on leaves under *in vivo* condition.

4.6.3.2 Detached leaf assay

The pathogenic variability among the eight isolates of *X. campestris* pv. *mangiferaeindicae* was studied by detached leaf technique *in vitro* condition on different varieties of mango.

The pathogenic variability among eight isolates of *X. campestris* pv. *mangiferaeindicae* bacteria *viz.,*Xcm1, Xcm2, Xcm3, Xcm4, Xcm5, Xcm6, Xcm7, and Xcm8 were studied *in vitro* by detached leaf technique under controlled conditions and leaves of total six varieties of mango *viz.,* Local, Kesar, Dasheri, Neelam, Amrapali and Alphanso were used.

Results (Table 4.9, Plate 4.8 and Fig. 4.5) revealed that, average incubation period (days) varied from 9.00 (Xcm1) to 16.33 (Xcm6) on different cultivars of mango among the eight isolates. However, average incubation `period (days) varied from 9.33 (Xcm3) to 11.33 (Xcm7) on Local cultivar of mango among eight isolates. Maximum incubation period (days) was in isolate Xcm7 (11.33) followed by Xcm4 (11.13), Xcm15 (10.66), followed by two isolates *viz.,* Xcm6 and Xcm8 exhibited same incubation period *i.e* 10.33, followed by Xcm2 (10.00), and Xcm1 (9.99), while minimum average incubation period (days) was in Xcm3 (9.33).

Table 4.9: Pathogenic variability among the *Xcm* isolates (detached leaf assay)

SN	Isolates	Agro-climatic Zones	Reaction on leaves of different cultivars after 20 days of inoculation											
			Local		Kesar		Dasheri		Neelam		Amrapali		Alphanso	
			Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms
1.	Xcm1	SC	9.99	+++	9.00	+++	10.66	++	12.66	+	14.33	+	15.66	+
2.	Xcm2	AR	10.00	++	10.33	++	12.33	+	12.33	+	14.66	+	15.33	+
3.	Xcm3	AR	9.33	+++	9.66	+++	12.66	++	13.33	+	13.99	+	14.99	+
4.	Xcm4	AR	11.13	++	10.00	+++	13.11	+	13.66	+	13.66	+	15.66	+
5.	Xcm5	AR	10.66	++	9.33	+++	11.66	+	14.11	+	14.33	+	16.13	+
6.	Xcm6	AR	10.33	+++	9.66	+++	12.99	+	11.99	+	13.66	+	16.33	+
7.	Xcm7	SC	11.33	++	10.33	++	13.33	+	14.333	+	14.13	+	15.11	+
8.	Xcm8	SC	10.33	+++	10.66	++	13.66	+	14.66	+	14.99	+	14.99	+

- No symptoms of leaf spot, + less symptoms of leaf spot, ++ moderate symptoms of leaf spot, +++ more symptoms of leaf spot

Plate 4.8. Pathogenic variability (detached leaf assay

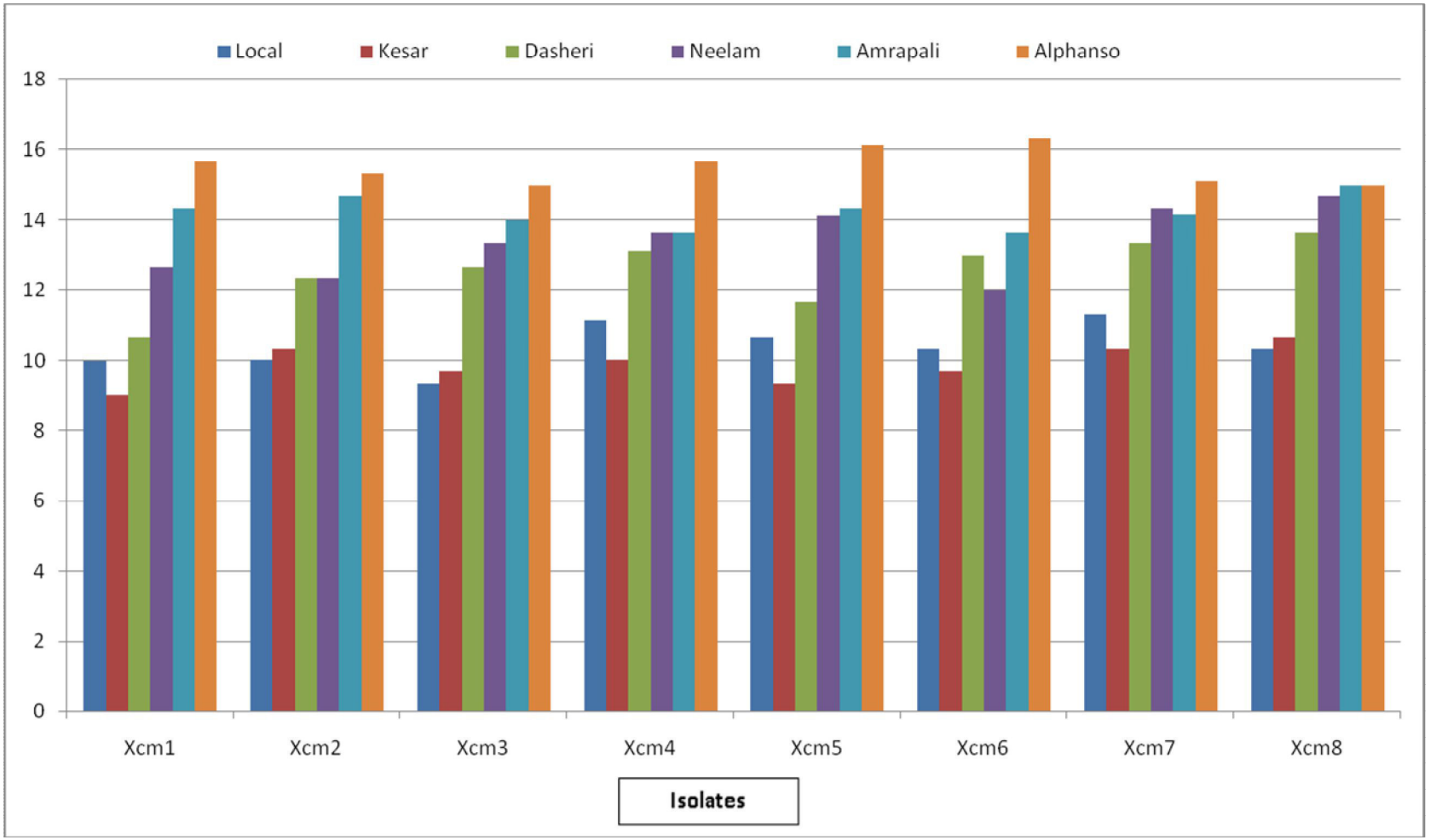


Fig. 4.5. Pathogenic variability among the *Xcm* isolates on different cultivars of mango (detached leaf method)

On Kesar cultivar of mango among eight isolates, maximum incubation period (days) was in isolate Xcm8 (10.66) followed by two isolates *viz.,* Xcm2 and Xcm7 exhibited same incubation period *i.e* 10.33, followed by Xcm4 (10.00) while, minimum average incubation period (days) was in Xcm1 (9.00) followed by Xcm5 (9.33), followed by two isolates *viz*., Xcm3 and Xcm6 again exhibited same incubation period (9.66).

On Dasheri cultivar of mango among eight isolates, maximum incubation period (days) exhibited by Xcm8 (13.66) followed by Xcm7 (13.33), Xcm4 (13.11), Xcm6 (12.99), Xcm3 (12.66) while, minimum average incubation period (days) was in Xcm1 (10.66) followed by Xcm2 (12.33) and Xcm5 (11.66).

On Neelam cultivar of mango among eight isolates, maximum incubation period (days) was in Xcm8 (14.66) followed by Xcm7 (14.33), Xcm5 (14.11), Xcm4 (13.66), Xcm3 (13.33) while, minimum average incubation period (days) was in Xcm6 (11.99) followed by Xcm2 (12.33) and Xcm1 (12.66).

On Amrapali cultivar of mango among eight isolates, maximum incubation period (days) was in isolates Xcm8 (14.99) followed by Xcm2 (14.66), followed by two isolates *viz.,* Xcm1 and Xcm5 *i.e* 14.33, followed by Xcm7 (14.13), Xcm3 (13.99) while, minimum average incubation period (days) was in two isolates *viz.,* Xcm4 and Xcm6 *i.e* 13.66.

On Alphanso cultivar of mango among eight isolates, maximum incubation period (days) was in isolate Xcm6 (16.33), followed by Xcm5 (16.13), followed by two isolates exhibited same incubation period *viz.,* Xcm1 and Xcm4 *i.e* 15.66, followed by Xcm2 (15.33) while minimum incubation period (days) was in two isolates Xcm3 and Xcm8 exhibited same incubation period *i.e* 14.99 followed by Xcm7 (15.11) and Xcm2 (15.33).

Symptoms exhibited by different isolates were grouped under four categories viz., (-) no leaf spot, (+) less leaf spot, (++) moderate leaf spot and (+++) more leaf spot. Different pattern of symptom expression was found in all the isolates among six cultivars. Kesar and Local cultivar of mango showed moderate to severe

bacterial leaf spot, while rest all cultivars *viz.,* Dasheri, Neelam, Amrapali and Alphanso showed lesser leaf spots.

From the ongoing results it has been inferred that the mango bacterial incubation period, frequency of spots and size varied from different cultivars due to isolates collected from different regions of Marathwada falling under three agro-climatic zones, it may be due to difference in virulence of isolates.

Similar results were also reported earlier by many workers. (Moffett *et al.,* 1977; Tsuchiya *et al.,* 2003; Sain *et al.,* 2008; Al-Saleh *et al.,* 2014). Dayakar and Gnanamanickam (1996) studied the pathogenic variability among 20 isolates collected from various locations of Southern India on six local commercial mango varieties and the bacterial suspension was artificially inoculated on mango leaves. The lesion size was measured after 10 days of inoculation and some strains were more aggressive on susceptible varieties. Tsuchiya *et al.,* (2003) tested the pathogenecity of *X. campestris* pv. *mangiferaeindicae* on detached leaves of mangos. Detached leaves were aseptically inoculated with 50 µl inoculum (10^8 cfu/ml) and were placed at 25^0 C under fluorescent light. Symptom development were observed after a week of inoculation. Sanahuja *et al.,* (2016) proved the pathogenecity of *X. campestris* pv. *mangiferaeindicae* strains by infiltration methods. Where mango varieties Kiett and Haden plants were injected with 18 hrs. old YGPA colonies ($1x10^5$ cfu/ml). After seven days, black lesions developed on inoculated leaves.

4.6.4. Morpho-cultural characters

Cultural characteristics *viz.,* pigmentation, colony shape, elevation, margin, surface appearance and cell shape of different eight test isolates were studied using NA as basal culture medium. Cell shape was observed using binocular microscope (400X).

Results (Table 4.10, Plate 4.9) revealed that, of the eight isolates tested, four isolates *viz.,* Xcm1, Xcm4, Xcm5 and Xcm7 exhibited creamy white pigmentation, Xcm2 and Xcm6, exhibited Creamy white with pale yellow ting pigmentation while rest two isolates *viz.,* Xcm3 and Xcm8 exhibited creamy white to pale yellow pigmentation . All the isolates had filiform colony shape, convex

elevation and six isolates viz., Xcm1, Xcm3, Xcm5, Xcm6 and Xcm8 had entire colony margin and rest three isolates Xcm2, Xcm4 and Xcm7 had irregular margin.

Table 4.10: Morphological and cultural characteristics of *X. campestris* pv. *mangiferaeindicae* isolates

Sr. No.	Isolates	Pigmentation	Elevation	Margin	Surface	Cell shape	Cell size (W x L µm)
1	Xcm1	Creamy white	Convex	Entire margin	Smooth and glistening	Single rods	0.4 x 1.1
2	Xcm2	Creamy white with pale yellow sting	Convex	Irregular margin	Smooth and glistening	Single rods	0.4 x 1.0
3	Xcm3	Creamy white to pale yellow	Convex	Entire margin	Smooth and glistening	Single rods	0.5x 1.2
4	Xcm4	Creamy white	Convex	Irregular margin	Smooth and glistening	Single rods	0.6 x 1.3
5	Xcm5	Creamy white	Convex	Entire margin	Smooth and glistening	Single rods	0.5 x 1.1
6	Xcm6	Creamy white with pale yellow sting	Convex	Entire margin	Smooth and glistening	Single rods	0.4 x 1.2
7	Xcm7	Creamy white	Convex	Irregular margin	Smooth and glistening	Single rods	0.5 x 1.3
8	Xcm8	Creamy white to pale yellow	Convex	Entire margin	Smooth and glistening	Single rods	0.5 x 1.1

Similar results were also reported earlier by many workers. (Ah-You *et al.,* 2007; Khalid and Sinha, 2008; Singh and Thind, 2014; Kharde *et al.,* 2018). Manicom and Wallis (1984) described that all strains isolated of *X. campestris* pv. *mangiferaeindicae* were rod shaped and motile by means of single polar flagellum. On nutrient agar the cell was 0.4 to 0.5 by 1.0 to 1.1 µm round, shallowly, convex colonies with entire margins were formed. The colour was initially smoke gray but soon became white to cream. With the age, the colonies became pale yellowish brown. Thiruamlesh (2012) reported that isolates of *X. campestris* pv. *mangiferaeindicae* were motile and small rods singly or in pairs and produced white to yellow, mucoid, circular, raised 1 to 2 mm colonies on YNA and NA medium.

Bandi (2019) studied cultural variability of eight isolates of *X. campestris* pv. *mangiferaeindicae*. The results revealed that the isolates differed with respect to colony characters such as size, shape, colour and appearance. Xcm1, Xcm2 and Xcm5 developed small to medium bacterial colonies and Xcm3, Xcm4, Xcm7 and Xcm8 developed medium to large colonies. The isolates Xcm1, Xcm3, Xcm5, Xcm6, Xcm7 and Xcm8 were having circular to irregular shape. Xcm2 and Xcm4 were having circular in shape. Isolates Xcm1, Xcm2, Xcm5 and Xcm7 were creamish white and isolates Xcm3, Xcm4, Xcm6 and Xcm8 formed creamish colonies.

4.6.5. Biochemical tests

Biochemical characters of *Xanthomonas campestris* pv. *mangiferaeindicae* were studied by subjecting the bacterial isolates to various biochemical tests *viz.,* gram staining, potassium hydroxide (KOH) solubility test, catalase test, starch hydrolysis test, tetrazolium salt tolerance test, anaerobic growth test, gelatin liquefication test, casein hydrolysis and xanthum gum production test.

Results (Table 4.11, Plate 4.10 (A & B)) revealed that, all the isolates were negative to gram reaction, anaerobic growth test and tetrazolium salt tolerance test while, positive to catalase oxidation test, KOH test, starch hydrolysis test, gelatin liquefication test, casein hydrolysis test and xanthum gum production. The results obtained are being narrated herein as follows.

Table 4.11: Biochemical characteristics of *Xanthomonas campestris* pv. *Mangiferaeindicae* isolates

Sr. No.	Isolates	Gram staining	Potassium hydroxide (KOH) solubility test	Catalase test	Starch hydrolysis test	Anaerobic growth test
1	Xac1	-ve	+ve	+ve	+ve	-ve
2	Xac2	-ve	+ve	+ve	+ve	-ve
3	Xac3	-ve	+ve	+ve	+ve	-ve
4	Xac4	-ve	+ve	+ve	+ve	-ve
5	Xac5	-ve	+ve	+ve	+ve	-ve
6	Xac6	-ve	+ve	+ve	+ve	-ve
7	Xac7	-ve	+ve	+ve	+ve	-ve
8	Xac8	-ve	+ve	+ve	+ve	-ve

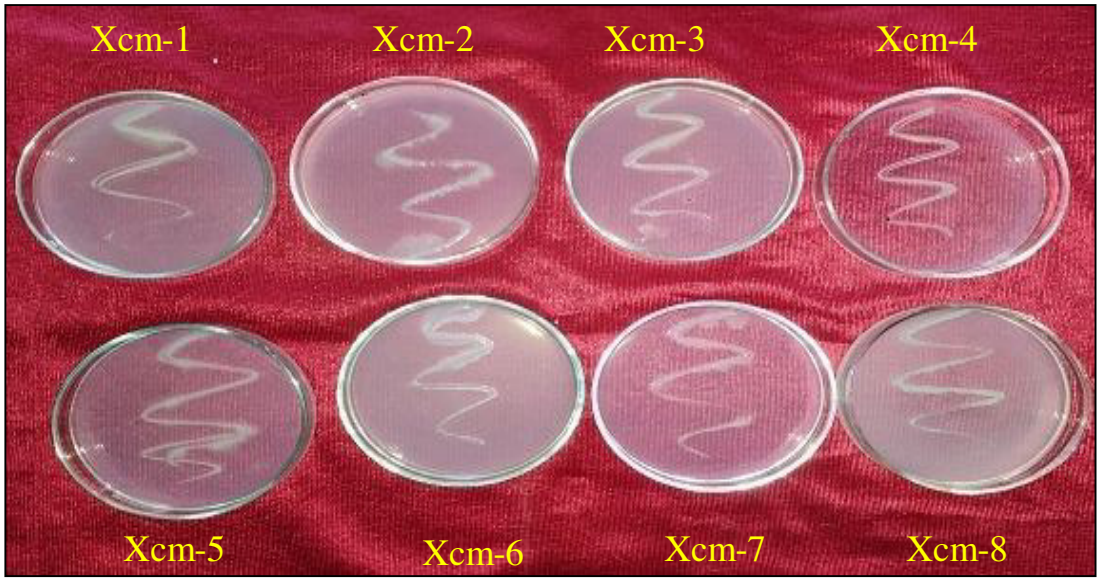
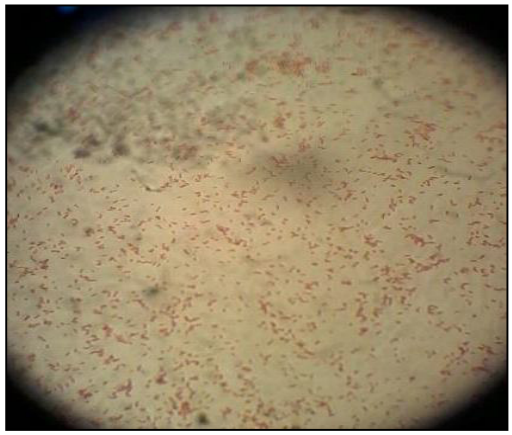
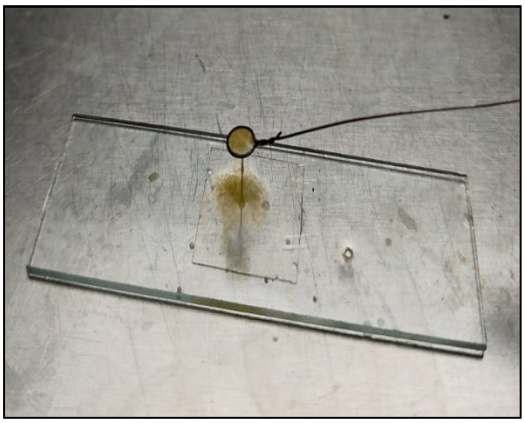


Plate 4.9. Morphocultural variability among the test isolates of *X. campestris* pv. *mangiferaeindicae*



Gram staining



KOH test

Catalase test

Starch hydrolysis test

Plate 4.10(A). Biochemical variability among the test isolates of *X. campestris* pv. *mangiferaeindicae*

Table 4.12: Biochemical characteristics of *Xanthomonas campestris* pv. *Mangiferaeindicae* isolates

Sr. No.	Isolates	Gelatin liquefication	Casein hydrolysis	Xanthum gum production	Tetrazolium salt tolerance test
1	Xac1	-ve	+ve	+ve	-ve
2	Xac2	-ve	+ve	+ve	-ve
3	Xac3	-ve	+ve	+ve	-ve
4	Xac4	-ve	+ve	+ve	-ve
5	Xac5	-ve	+ve	+ve	-ve
6	Xac6	-ve	+ve	+ve	-ve
7	Xac7	-ve	+ve	+ve	-ve
8	Xac8	-ve	+ve	+ve	-ve

4.6.5.1. Gram staining

Microscopic examination of Gram's stained *Xanthomonas campestris* pv. *mangiferaeindicae* mount elucidated that the test bacterium did not retained violet colour of the primary stain (crystal violet) but cells appeared pink coloured due to counter staining with stain safranin. Hence the test bacterium was gram negative, straight rods which is characteristic feature of plant pathogenic bacteria (Table 4.11, Plate 4.10 (A)).

4.6.5.2. Potasium hydroxide test (KOH)

Formation of slime threads or loop is an indication of being gram negative. The present study (Table 4.11, Plate 4.10 (A)) revealed that, the test bacterium showed positive reaction to KOH test.

4.6.5.3. Catalase test

Results (Table 4.11, Plate 4.10 (A)) revealed that, the test bacterium produced gas bubbles when mixed on glass side with a few drops of 3% hydrogen peroxide, which indicated positive catalase test.

4.6.5.4. Starch hydrolysis

Results revealed that (Table 4.11, Plate 4.10(A)) the test bacterium produced colourless zone around bacterial growth on starch agar medium flooded with lugol's iodine and showed positive for starch hydrolysis test. The test bacterium

is hydrolyzed starch by exoenzyme amylase and broken down to dextrins, maltose and glucose / alpha-amylase.

4.6.5.5. Anaerobic growth test

Anaerobic growth test is differential test for aerobic and non-aerobic bacteria. In this experiment al eight isolates tested and gave negative anaerobic activity (Table 4.11).

4.6.5.6. Gelatin liquification test

Results revealed that (Table 4.12, Plate 4.10 (B)), in the test tube inoculated with test bacterium, liquification of gel, column was observed and no liquification of gel was recorded in control. Hence the test bacterium showed positive reaction to gelatin liquification.

4.6.5.7. Casein hydrolysis test

Results (Table 4.12, Plate 4.10 (B)) revealed that, the test bacterium degraded the protein casein by producing protieolytic exo-enzymes by growing the isolates on milk agar plates. Colourless zone was observed around the bacterial growth which indicates the hydrolysis of casein, which showed all isolates gave positive reaction to casein hydrolysis test.

4.6.5.8. Xanthum gum production test

Xanthum gum, an extracellular bacterial polysaccharide composed by glucose, mannose and glucornic acid was observed greatly around the colonies which was cream coloured pigment. Which showed all isolates gave positive reaction to xanthum gum production test (Table 4.12, Plate 4.10 (B)).

4.6.5.9. Tetrazolium salt tolerance test

Xanthomonads are extremely sensitive to tetrazolium salts at 0.1% concentration of this salt, no isolates out of eight able to grow. However, the results were similar at 0.02% concentration of tetrazolium salt which proved that all the isolates were extremely sensitive to tetrazolium salt (Table 4.12).

Gelatin liquefication

Caesin hydrolysis

Xanthum gum production

Plate 4.10(B). Biochemical variability among the test isolates of *X. campestris* pv. *mangiferaeindicae*

Results of some of these biochemical characteristics deployed in the present investigation were in accordance with the results obtained by McGuire (1988), Yasuhara *et al.,* (2012), Silva *et al.,(*2017), Bhure *et al.,* (2019). Yasuhara (2012) reported that strains of *X. campestris* pv. *mangiferaeindicae* were non pigmented, gram negative, oxidative, positive for both starch and esculin hydrolysis, negative for nitrate reduction. Nijafipour et al., (2014) identified *X. campestris* pv. *mangiferaeindicae* based on phenotypic and biochemical tests were small, white and yellow, slightly mucoid, gram-negative obligate aerobic colonies were observed on sucrose nutrient agar medium. All strains were oxidase negative, but catase, levan production, gelatin, starch and esculin hydrolysis, tween 80, litmus milk, hypersensitive reaction on geranium, hydrogen sulphide production tests were positive. Bandi (2019) studied biochemical characteristics of the eight isolates *X. campestris* pv. *mangiferaeindicae*. The results revealed that bacterium liquefied the gelatin, hydrolyzed the starch and casein, positive for KOH and catalase tests and produced xanthum gum.

4.6.6. Molecular variability

4.6.6.1. Molecular characterization of *X. campestris* pv. *mangiferaeindicae* by using RAPD analysis:

4.6.6.1.1. DNA Extraction:

The eight isolates of Xanthomonas as described in Table 4.5. , were grown overnight at 28ºC on nutrient broth following the method described by Adachi and Takashi (2002), with some modification for isolation of DNA. The purity of DNA was checked by spectroscopic analysis at OD 260-280 nm. The OD 260:280 ratio was 1.8, indicating that DNA was sufficiently pure and free from contamination.

The good quality of DNA was assessed, by resolving on 0.8% agarose gel. Similarly, the quantification of DNA was done by comparing DNA samples with known amount of DNA i.e. lambda DNA. The concentration of DNA of all *Xanthomonas* isolates was found in the range between 70-100 ng /µl. These DNA sample were diluted with sterile water and used in molecular characterization study.

Similarly, the standardized protocol of DNA extraction described by Sambrook *et al.,* (1989) has modified by Boom *et al.,* (1990) and used to isolate DNA from bacteria. Also the genomic DNA of species *Xanthomonas* and *Pseudomonas* have been isolated by several researchers, Luiz Humbert *et al.,* (2000), Adachi and Takashi (2002) and Numes *et al.,* (2008).

4.6.6.1.2. Confirmation of *Xanthomonas* by using PCR:

A) Standardization of PCR Protocol

The PCR protocol described by Williams *et al.,* (1990) was used with some modifications by using genus specific diagnostic primer for confirmation of eight isolates of *Xanthomonas*. The PCR amplification reaction was optimized by varying concentration of PCR components. Further, PCR products finally hold at 4^0 C. PCR amplified product was separated by electrophoresis on 1.2 % agarose gel, stained with Ethidium bromide and visualized under gel documentation System. These PCR products were found ~420 to 450bp amplicon size (Plate 4.11).

4.6.6.2. Diversity analysis using RAPD marker

The genomic DNA of all *Xanthomonas* was subjected for PCR amplification by using RAPD primers from operon tech, USA (Eurofins). Initially 20 set of primers were screened with eight strains of *Xanthomonas* among which five primers were produced larger no. and reproducible amplicons and further those were employed for molecular characterization of eight *Xanthomonas*.

Table 4.13. List of RAPD primers used and the number of total polymorphic bands, produced in *Xanthomonas* strains

Primer	Sequence (5'-3')	Total No. of Bands	Total No. of Polymorphic Bands	Total No. of Monomorphic Bands	Total No. of fragment amplified	Polymorphic Percentage (%)
OPB-06	TGCCGAGCTG	16	16	0	67	100.00
OPB-16	AGGGGTCTTG	5	4	1	25	80.00
OPB-17	GAAACGGGTG	12	12	0	41	100.00
OPB-19	TGCAGTTTGA	8	8	0	34	100.00
OPB-20	TCGGCGATAG	8	8	0	37	100.00
Total		**49**	**48**	**1**	**204**	**480.00**
Mean		**9.80**	**9.60**	**0.20**	**40.80**	**96.00**

Results (Table 4.13, Plate 4.12 (A, B & C) and Fig. 4.6) revealed that the average sizes of amplicons generated by all RAPD primer were found in the range

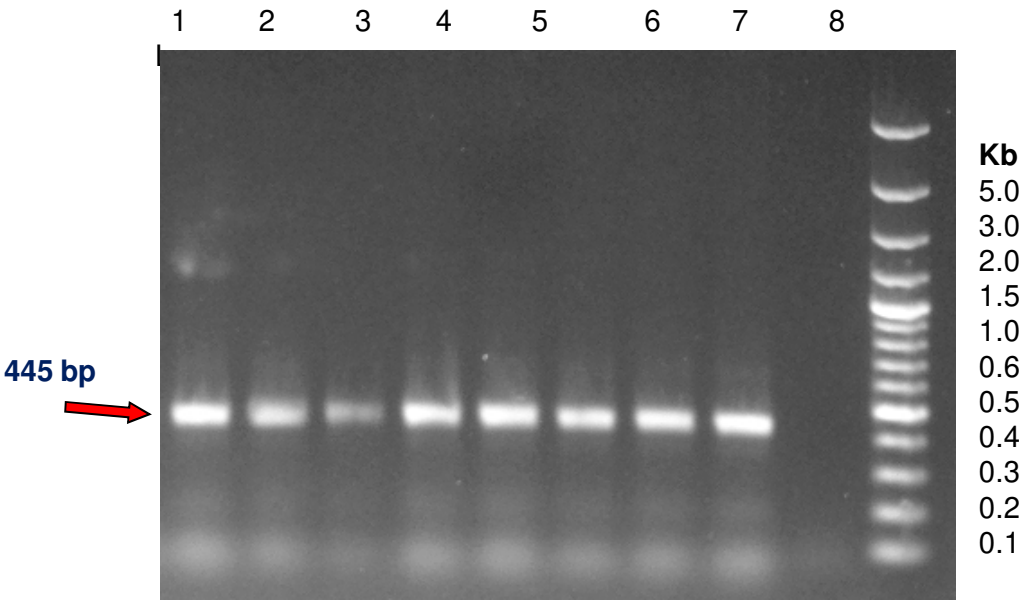


Plate 4.11. Confirmation of strains of *Xanthomonas campestris* pv. *mangiferaeindicae* isolates by using primer genus specific primer (*Xanthomonas*): Lane L, Marker (1 Kb DNA ladder); Lanes 1–8 strains of *Xanthomonas campestris* pv. *mangiferaeindicae*

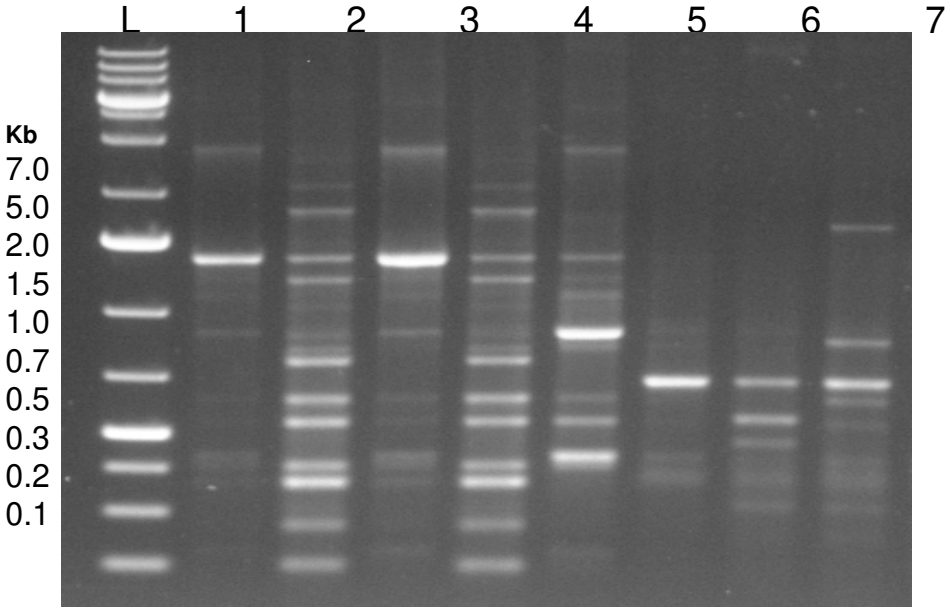


Plate 4.12(A). RAPD fingerprint profile of 8 isolates of *Xanthomonas* by using primer OPB-06: Lane L, Marker (1Kb DNA ladder); Lanes 1 –8 isolates of *Xanthomonas*

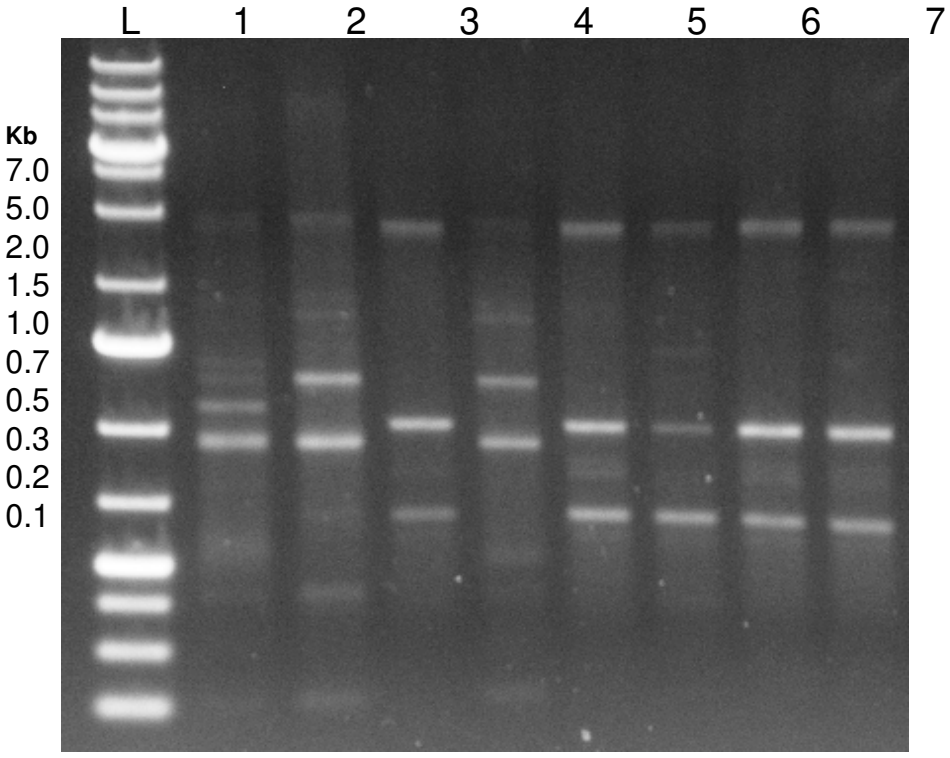


Plate 4.12(A). RAPD fingerprint profile of 8 isolates of *Xanthomonas* by using primer OPB-16: Lane L, Marker (1Kb DNA ladder); Lanes 1 –8 isolates of *Xanthomonas*

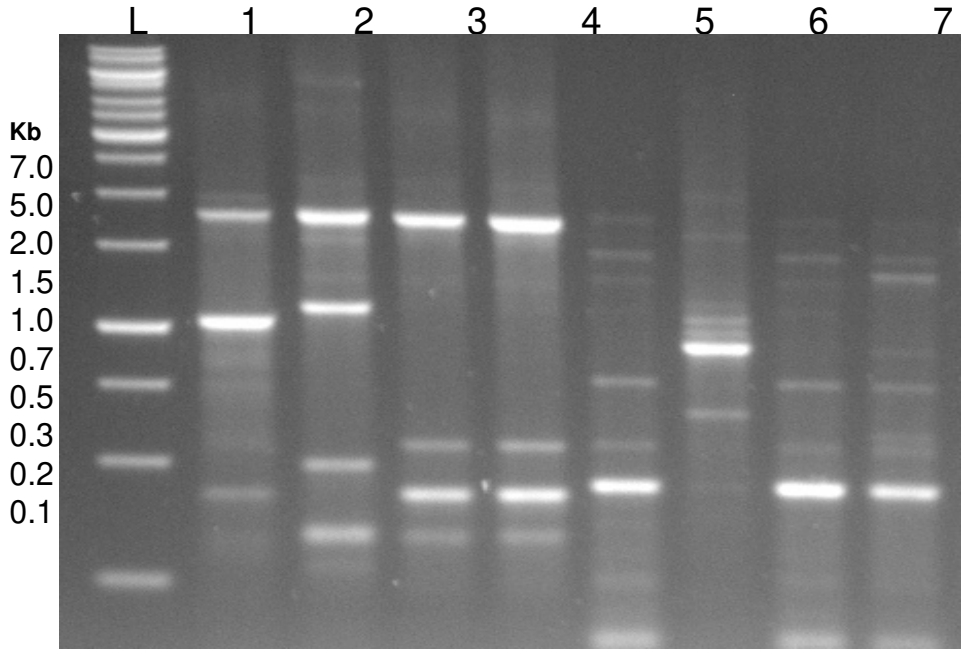


Plate 4.12(B). RAPD fingerprint profile of 8 isolates of *Xanthomonas* by using primer OPB-17: Lane L, Marker (1Kb DNA ladder); Lanes 1 –8 isolates of *Xanthomonas*

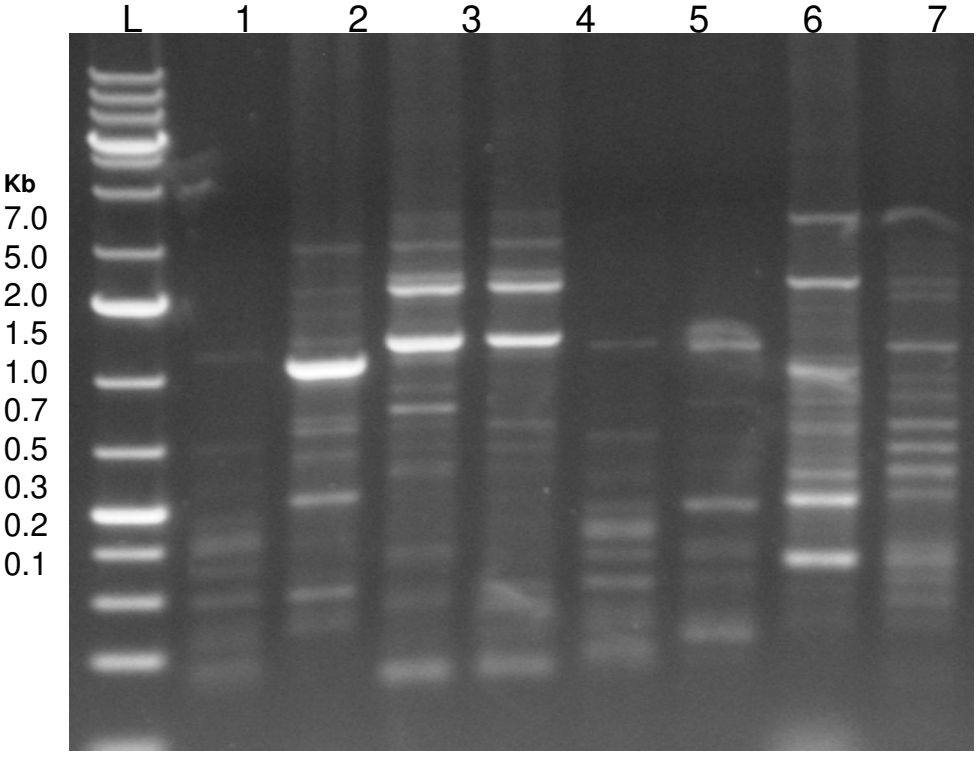


Plate 4.12(B). RAPD fingerprint profile of 8 isolates of *Xanthomonas* by using primer OPB-19: Lane L, Marker (1Kb DNA ladder); Lanes 1 –8 isolates of *Xanthomonas*

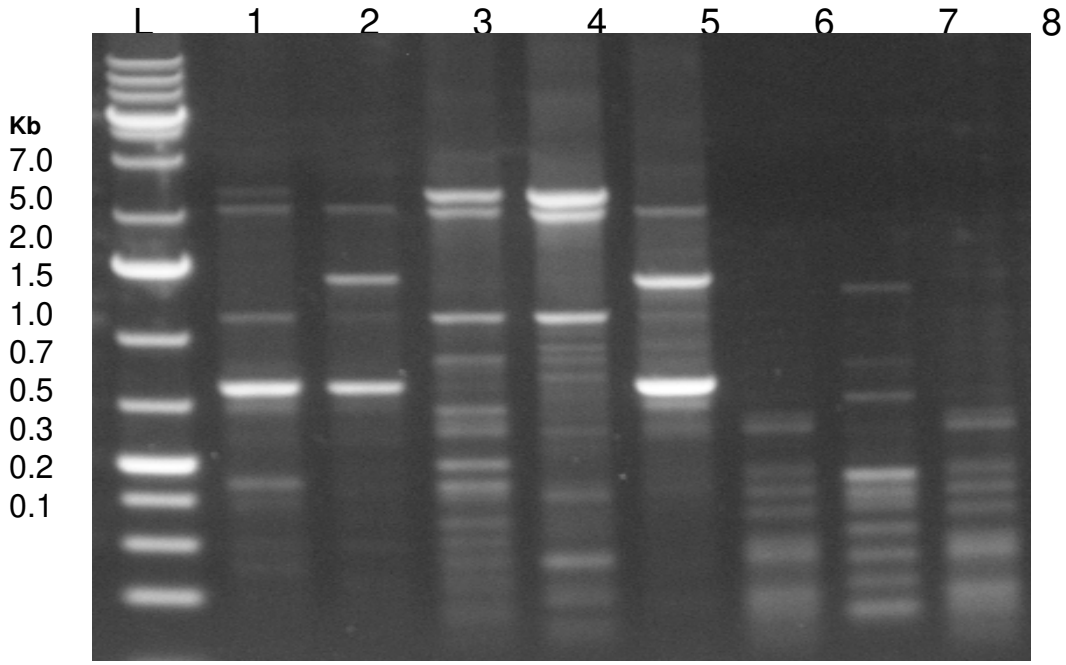


Plate 4.12(C). RAPD fingerprint profile of 8 isolates of *Xanthomonas* by using primer OPB-17: Lane L, Marker (1Kb DNA ladder); Lanes 1 –8 isolates of *Xanthomonas*

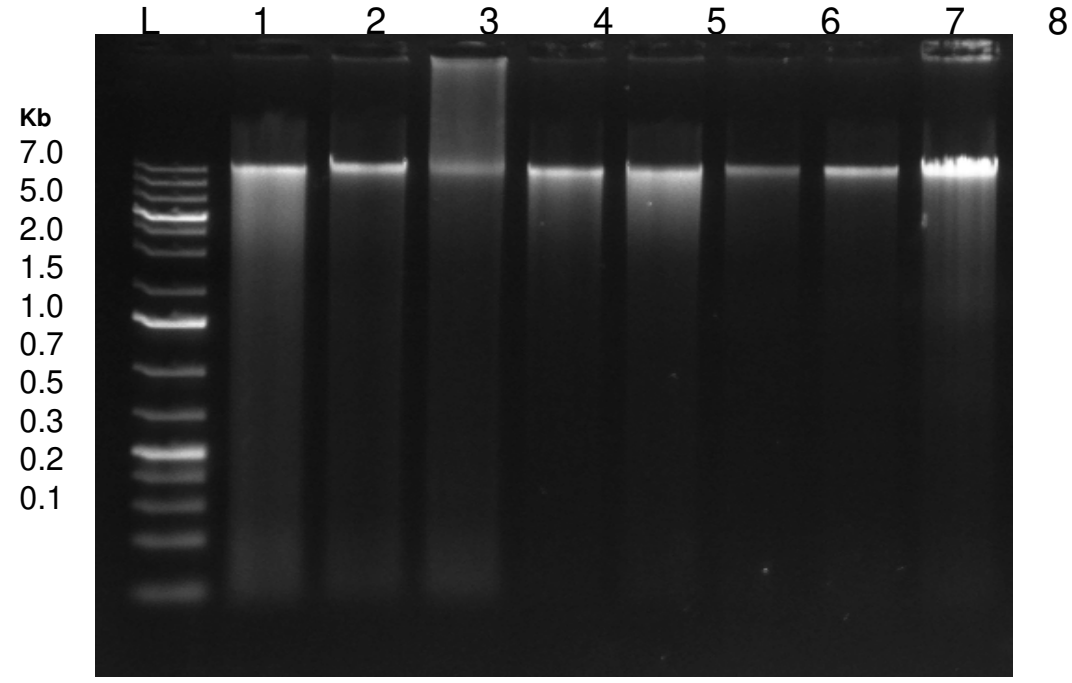


Plate 4.12(C). DNA isolation of 8 isolates of *Xanthomonas* using SDS method: Lane L, Marker (1Kb DNA ladder); Lanes 1 –8 isolates of *Xanthomonas*

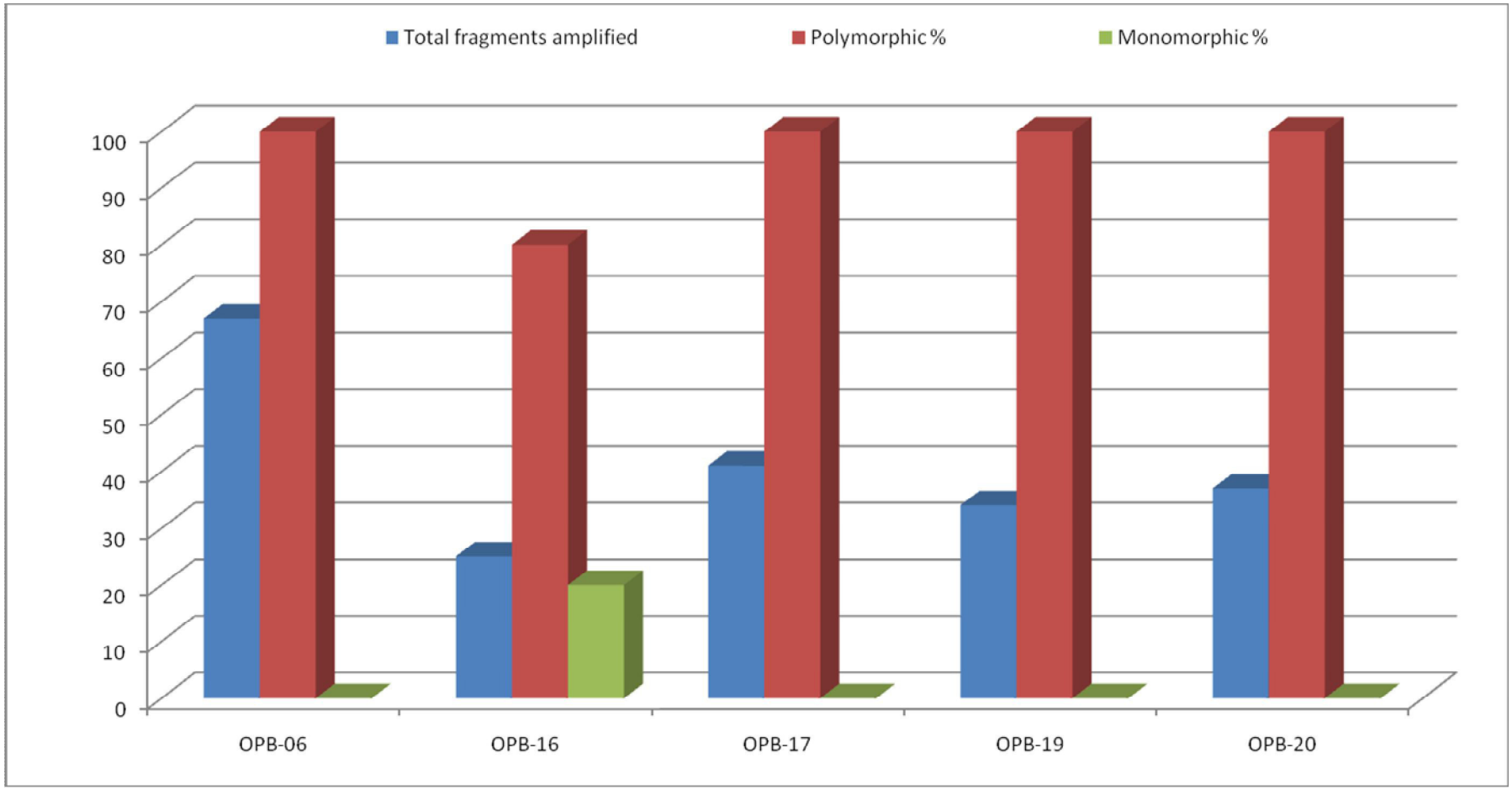


Fig. 4.6. Polimorphism generated during fingerprint analysis of eight isolates of *X. campestris* pv. *mangiferaeindicae*

between 100 bp to 10.0 kb. The RAPD-PCR amplification result showed that five RAPD primers generated total 49 bands, out of these, 48 bands were found polymorphic and one band was reported as monomorphic with an average of 0.20 bands per primers among eight isolates of *Xcm*. The primer OPB-06, OPB-17, OPB-19 and OPB-20 were more found informative as they could generate total number of 16, 12, 8, and 8 bands respectively. The primer OPB-16 has generated lowest number of bands *i.e.* 5. The primer OPB-06 has produced higher number of amplicons i.e. 67, followed by OPB-17 which has produced 41 amplicons. While the primer OPB-16 has produced minimum number of amplicons *i.e.* 25.

Overall all five primers were generated total 204 amplicons with an average of 40.80 amplicons per primers. Out of 204 amplicons, 196 amplicons were found polymorphic, they showed 96.00 % polymorphism. Similarly, 8 amplicons were found monomorphic. They showed, 4.00 % monomorphism and the average number of monomorphic amplicons per primer were 0.20.

4.6.6.3. Cluster analysis of RAPD DNA fingerprint

The data obtained by RAPD markers was analyzed by NTSYS pc2.02i and dendrogram was dipicted by using Jaccard's similarity coefficient. The genetic similarity matrix obtained by Jaccard's similarity coefficient of the *Xanthomonas* isolates has described in Table 4.14.

Table 4.14. The similarity matrix representing coefficient of similarity among eight isolates of *X. campestris* pv. *mangiferaeindicae* on RAPD marker

	Xcm-1	**Xcm-2**	**Xcm-3**	**Xcm-4**	**Xcm-5**	**Xcm-6**	**Xcm-7**	**Xcm-8**
Xcm-1	1.000	0.536	0.696	0.623	0.565	0.565	0.420	0.536
Xcm-2	0.536	1.000	0.551	0.797	0.565	0.420	0.420	0.536
Xcm-3	0.696	0.551	1.000	0.6966	0.522	0.406	0.580	0.667
Xcm-4	0.623	0.797	0.696	1.000	0.478	0.362	0.507	0.594
Xcm-5	0.565	0.565	0.522	0.478	1.000	0.623	0.652	0.623
Xcm-6	0.565	0.420	0.406	0.3362	0.623	1.000	0.565	0.594
Xcm-7	0.420	0.420	0.580	0.507	0.625	0.565	1.000	0.739
Xcm-8	0.618	0.603	0.639	0.632	0.629	0.567	0.611	0.661
Avg. Similarity	0.618	0.603	0.639	0.632	0.629	0.567	0.611	0.661

Dendrogram generated based on UPGMA analysis of RAPD data grouped all these isolates in to two major clusters (Figure 4.7). These clusters were formed on the basis of genus as well as species level. The group I, comprised, maximum of four isolates together which showed 56.40 % genetic similarity *viz.*, Xcm1, Xcm2, Xcm3 and Xcm4. The cluster I comprised isolates of Xcm-2 and Xcm-4 showed that higher similarity each other i.e. 80 %. Cluster II comprised four isolates together showing 57.2 % similarity with each other viz., Xcm-5, Xcm-06, Xcm-07 and Xcm-08. Theses cluster comprised fungal isolates of Xcm-07 and Xam-08 showed that higher similarity each other i.e. 75 %. The cluster I and II comprised of eight isolates showed that 51 % similarity each other.

RAPD analysis is accepted to be a proven tool to distinguish variability between organisms or even between different strain and isolates of same organisms (Henson and French, 1997). Similarly several researchers have exploited this technique and discriminated various pathogens at genus as well as species.

4.6.7. Effect of different age of leaf on disease development

Table 4.15: Effect of different age of leaf on disease development (On detached leaves)

Sr. No.	Isolate	Age of the leaf	No. of days required for initiation of symptoms*	No. of spots observed after inoculation*	Symptoms
1	Xcm1	Young	No symptoms	-	-
		Mid age	11.66	2	Water soaked circular to irregular, brown spots
		Old	9.33	3.66	Water soaked irregular, brown spots with yellow halo
2	Xcm2	Young	No symptoms	-	-
		Mid age	12.33	2.33	Water soaked circular to irregular, brown spots
		Old	11.99	3.33	Water soaked circular to irregular, brown spots
3	Xcm3	Young	No symptoms	-	-
		Mid age	12	1.66	Water soaked circular to irregular, brown spots
		Old	11.66	2.66	Water soaked circular to irregular, brown spots with yellow halo

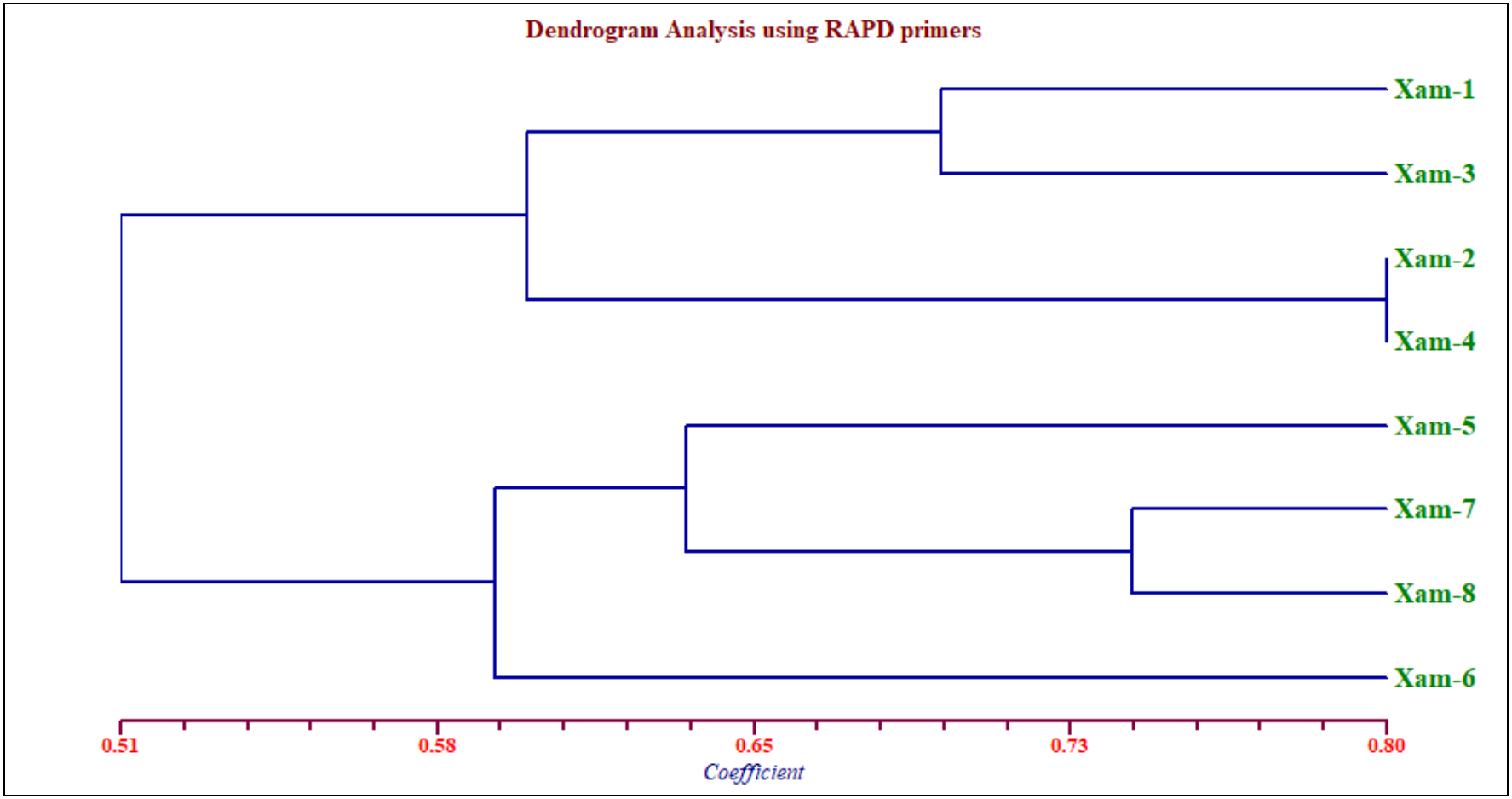


Fig. 4.7. Dendrogram based on RAPD analysis depicted relationship between eight isolates of *X. campestris* pv. *mangiferaeindicae*

4	Xcm4	Young	No symptoms	-	-
		Mid age	13.13	2	Water soaked, brown to dark brown spots
		Old	10.33	2.66	Water soaked, brown to dark brown spots with yellow halo
5	Xcm5	Young	No symptoms	-	-
		Mid age	12	1.66	Water soaked, brown to dark brown spots with yellow halo
		Old	11	2.33	Water soaked, circular to irregular, dark brown spots with yellow halo
6	Xcm6	Young	No symptoms	-	-
		Mid age	12.13	2.33	Water soaked, irregular, brown spots with yellow halo
		Old	11.33	3	Water soaked, irregular, dark brown spots with yellow halo
7	Xcm7	Young	No symptoms	-	-
		Mid age	12.99	1.33	Water soaked, irregular, light brown spots
		Old	11.33	2	Water soaked, circular to irregular, dark brown spots with yellow
8	Xcm8	Young	No symptoms	-	-
		Mid age	11.66	1.66	Water soaked, irregular, dark brown spots
		Old	10.33	2.99	Water soaked, circular to irregular, dark brown spots with yellow

Results (Table 4.15, Plate 4.14 and Fig. 4.8 & 4.9) revealed that old age leaves were more susceptible than mid age and young age leaves. Old age leaf required minimum average days for initiation of symptoms (9.33 days) for isolate Xcm1 followed by Xcm4 (10.33 days), Xcm8 (10.33 days) whereas; maximum days for initiation of symptoms was 11.99 (Xcm2). Mid age leaf required minimum average days for initiation of symptoms (11.66 days) for isolate Xcm8 followed by Xcm3 (12 days), Xcm5 (12 days) whereas; maximum days for initiation of symptoms was 13.13 (Xcm4), whereas young leaf didn't show any symptoms. Old age leaves showed 2.00 to 3.66 average number of spots. Isolate Xcm1 showed maximum number of spots on old leaves followed by Xcm2 (3.33), Xcm6 (33.00), Xcm8 (2.99), followed by two isolates Xcm3 and Xcm4 (2.66) whereas; minimum average spots showed by Xcm7 (2.00) followed by Xcm5 (2.33). Mid age leaves showed 1.33 to

2.33 average number of spots. Xcm2 and Xcm6 showed maximum number of spots *i.e* 2.33 on mid age leaves followed by two isolates Xcm1 and Xcm4 (2.00) followed by again three isolates *viz.,* Xcm3, Xcm5 and Xcm8 (1.66) whereas; minimum average number of spots showed by Xcm7 (1.33). Symptoms observed on mid age leaves were water soaked circular to irregular, light to brown spots and on old age leaves were water soaked circular to irregular, dark brown spots with yellow halo. Studies revealed that the susceptibility of plant organs varies with the age. Young leaves were resistant, likely because they do not have functional stomata, whereas old leaves of mango were more susceptible to the bacterial leaf spot disease as they have maximum number of functional stomata.

Similar results were also reported earlier by many workers. Thind (2012) mentioned the susceptibility of mango plant parts varies with time for given cultivar. Young leaves were resistant, because they do not functional stomata. However they became very susceptible when they enlarge, with lesions appearing just after leaf hardening. Fruit susceptibility increases over time. This correlates with maximum receptivity of lenticels.

4.7. Host range

Table 4.16: Host range of *X. campestris* pv. *mangiferaeindicae* under *in vitro* conditions

Sr. No.	Common Name	Botanical Name	Family	Infectivity
1	Lemon	*Citrus limon*	Rutaceae	-
2	Sweet lime	*Citrus limetta*	Rutaceae	-
3	Pomegranate	*Punica granatum*	Punicaceae	-
4	Jamun	*Syzygium cumini*	Myrtaceae	-
5	Fig	*Ficus carica*	Moraceae	-
6	Almond	*Prunus dulcis*	Rosaceae	-
7	Gauva	*Psidium guajava*	Myrtaceae	-
8	Custard apple	*Annona reticulate*	Annonaceae	-
9	Mango	*Mangifera indica*	Anacardiaceaee	+

The results of artificial inoculation of plants with *X. campestris* pv. *mangiferaeindicae* (Table 4.16, Plate 4.13) showed that out of nine plants species

Plate 4.13. Host range of *X. campestris* pv. *mangiferaeindicae*

Before

After

Plate 4.14. Effect of leaf age on disease development

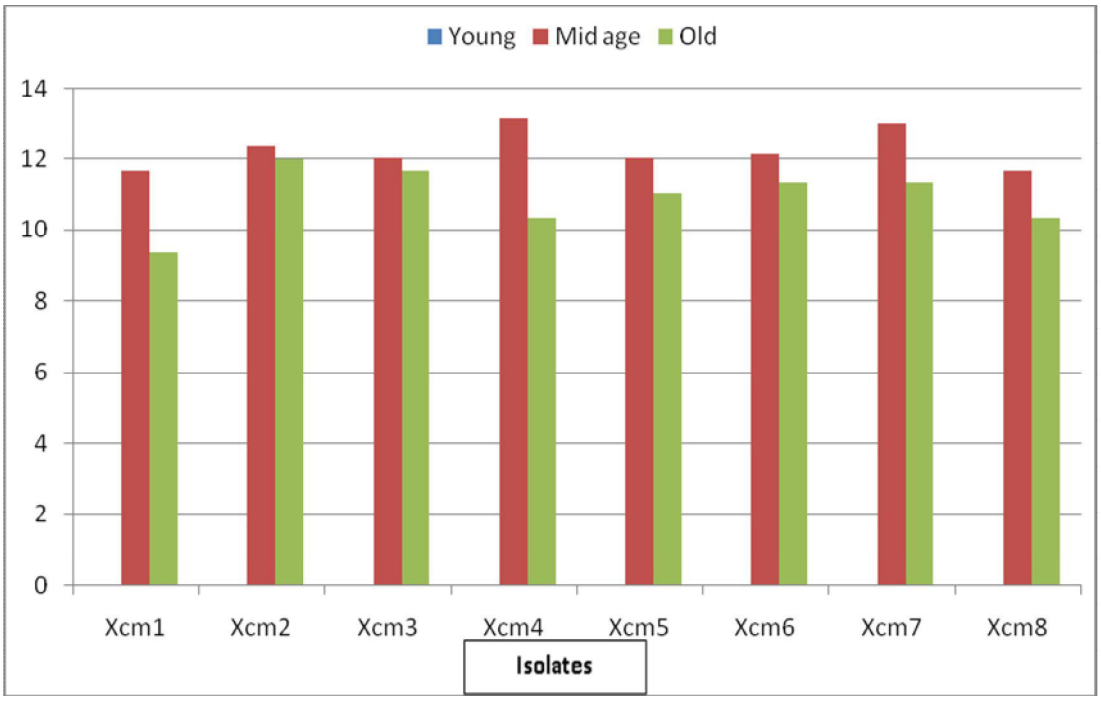


Fig. 4.8. Effect of different age of leaf on disease development (No. of days required for initiation of symptoms)

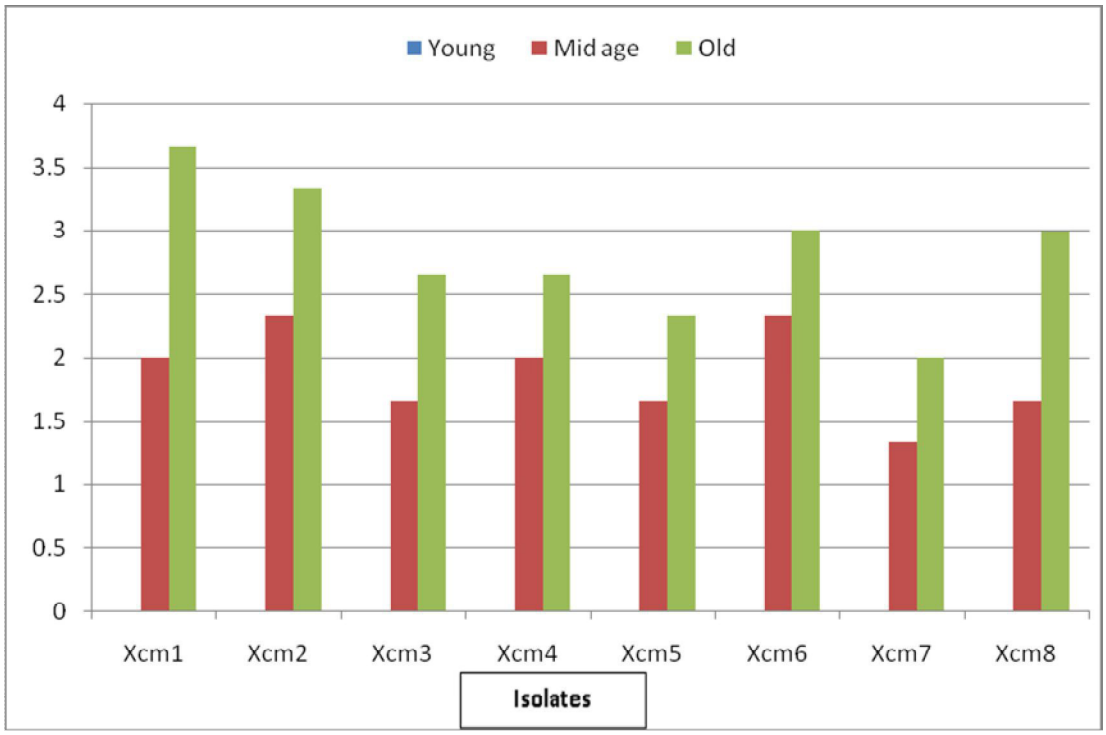


Fig. 4.9. Effect of different age of leaf on disease development (No. of spots observed after inoculation)

tested the bacterium failed to infect any of the fruit crops. These results revealed that the bacterium is a potential threat to infect the mango crop of Anacardiaceace family.

The results obtained in present study on host range were similar with the reports of earlier workers. Ravikumar and Khan (2000) studied the host range to assess the infection of *X. campestris* pv. *vesicatoria*. The results revealed that the three isolates of *X. campestris* pv. *vesicatoria* from tomato did not cause infection in other crop. Plants and weeds tested *viz.,* bean, bell papper, brinjal, chilli, potato cowpea, tobacco, *Datura stramonium* and *Solanum* sp. except its host *i.e* tomato. However, infection of *X. campestris* pv. *vesicatoria* on tomato were seen 20 days after inoculation. Bhat *et al.* (2010) studied host range under conditions of artificial inoculation. Results revealed that the bacterium infected almost all the crucifer crops cultivated in Kashmir, besides some cruciferous weeds but failed to infect any of the non-cruciferous plant species tested.

4.8. Epidemiological studies

4.8.1. Survival of *X. campestris* pv. *mangiferaeindicae*

Isolations of *X. campestris* pv. *mangiferaeindicae* were carried from infected leaves, twigs and barks collected from orchards with 15 days of interval the isolation procedure was repeated till the organism was found in pure form. It was found that pathogen survived in the leaves upto 3 to 4 months, 5 to 6.5 months on twigs and 8 to 9.5 months on barks of mango plant.

Results (Table 4.17) revealed that the bacterium isolated from the leaves declined their growth from 30th December onwards. Initially the CFU count on leaves was 1x10^{7} and it started declining from 31st October onwards. The growth of the bacterium on twigs declined from middle of March. Initially CFU count was 1x10^{6} on twigs and get started declining upto 1x10^{3} from 30th October onwards. While the growth of the bacterium on barks declined from middle of April. Initially CFU count was 1x10^{6} on barks and get started declining upto 1x10^{3} from 15th November onwards. Bacterial populations quickly decreases to undectable levels during dry conditions. From the results it was revealed that *X. campestris* pv. *mangiferaeindicae* survived more in twigs and barks than the leaves.

Table 4.17: Survival of *Xanthomonas campestris* pv. *mangiferaeindicae* on different plant parts of mango

Sr. No.	Date of isolation	Occurrence of pathogen			CFU/ml observed after isolation		
		Leaves	Twigs	Barks	Leaves	Twigs	Barks
1	1st September 2018	+	+	+	$1x10^7$	$1x10^6$	$1x10^6$
2	15th September 2018	+	+	+	$1x10^7$	$1x10^6$	$1x10^6$
3	1st October 2018	+	+	+	$1x10^7$	$1x10^6$	$1x10^6$
4	15th October 2018	+	+	+	$1x10^6$	$1x10^6$	$1x10^6$
5	30th October 2018	+	+	+	$1x10^6$	$1x10^5$	$1x10^6$
6	15th November 2018	+	+	+	$1x10^5$	$1x10^5$	$1x10^5$
7	30th November 2018	+	+	+	$1x10^4$	$1x10^5$	$1x10^5$
8	15th December 2018	+	+	+	$1x10^4$	$1x10^5$	$1x10^5$
9	30th December 2018	-	+	+	-	$1x10^5$	$1x10^5$
10	15th January 2019	-	+	+	-	$1x10^5$	$1x10^5$
11	30th January 2019	-	+	+	-	$1x10^4$	$1x10^5$
12	15th February 2019	-	+	+	-	$1x10^4$	$1x10^5$
13	2nd March 2019	-	+	+	-	$1x10^4$	$1x10^5$
14	16th March 2019	-	+	+	-	$1x10^4$	$1x10^5$
15	31st March 2019	-	-	+	-	$1x10^4$	$1x10^4$
16	15th April 2019	-	-	+	-	$1x10^3$	$1x10^4$
17	30th April 2019	-	-	+	-	$1x10^3$	$1x10^4$
18	15th May 2019	-	-	+	-	$1x10^3$	$1x10^3$
19	30th May 2019	-	-	+	-	$1x10^3$	$1x10^3$
20	15th June 2019	-	-	-	-	-	$1x10^3$

+ = pathogen occurred, - = pathogen not occurred

The results obtained in present study on survival of pathogen were similar with the reports of earlier workers. Khare and Khare (1995) reported *X. campestris* pv. *glycines* remained viable for 110 days in infected leaves, kept on soil surface, but when infected leaves were buried in the soil at 15 cm depth, the over withering period was reduced to 29 days. No survival of bacterium was found in infected leaves buried at 30 cm depth in sterilized soil and 160 days over withering period was found in wire house conditions. Gagnevin and Pruvost (2001) studied survival of *Xanthomonas campestris* pv. *mangiferaeindicae* was very efficient in leaf lesions. Bacterial population decreases relatively slowly in leaf lesions on trees maintained in controlled conditions. Three month old leaf lesions on susceptible

cultivar may contain up to 10^7 CFU and up to 10^5 CFU were detected in 18 month old lesions. Twig cankers also served as a source of inoculum, but their relative importance compared with bacterial inoculum in leaves was difficult to assess.

4.8.2. Effect of different temperature and leaf wetness durations on development of disease (on detached leaves)

Temperature and moisture plays a vital role in disease progress. Different temperature and leaf wetness durations were assessed to observe the disease progress on detached leaves of variety Kesar. The initial disease symptoms appeared at temperature of 25 ^{0}C, 30 ^{0}C and 35 ^{0}C after 48 hours of leaf wetness period with an average lesion size of 1.0 mm, 1.1 mm and 1.0 mm, respectively. However, at 40 ^{0}C temperature the symptoms appeared after 72 hours of inoculation with an average lesion size of 1.2 mm. No growth was observed at temperature levels of 5, 10, 15 and 20 ^{0}C at different leaf wetness durations of 3, 6, 12, 24, 48 and 72 hours after the inoculation of *X. campestris* pv. *mangiferaeindicae* (Table 4.18).

Table 4.18: Effect of different temperature and leaf wetness durations on development of disease (on detached leaves)

Temp ^{0}C / LWD (hrs)	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
3	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-
48	-	-	-	-	1.0 mm	1.1 mm	1.0 mm	-
72	-	-	-	-	1.1 mm	1.5 mm	1.8 mm	1.2 mm

LWD: Leaf wetness durations

4.8.3. Progress of disease under field conditions in the year 2018-19

In order to ascertain the role of environmental factors particularly maximum and minimum temperature, relative humidity and annual rainfall in disease development under field conditions, experiment was conducted at VNMKV,

Parbhani, during the year 2018-19 and 2019-20. The observations on maximum and minimum temperature, relative humidity, annual rainfall were recorded with the effect from 14th standard meteorological week when the fruit started to develop upto the 37th standard meteorological week. The data on disease incidence and disease severity on fruit and leaf were recorded at seven days interval and daily observations on meteorological factors were expressed at mean value for the period intervening the two data recording dates (7 days) and are presented in Table 4.19 and Fig. 4.10 & 4.11.

Table 4.19: Development of disease in relation to weather parameters under field conditions during, 2018-19

Sr. No.	SMW	Mean Temp (^{0}C)		Rainfall (mm)	Mean RH (%)	Fruit		Leaves	
		Max.	Min.			PDI	PDS	PDI	PDS
1.	14	39.9	19.9	0	52	0	0	0.21	0.12
2.	15	38.4	21.9	0	53	0	0	0.23	0.27
3.	16	40.9	22.4	0	48	0.13	0.11	0.69	0.59
4.	17	41.9	20.9	0	41	0.67	0.71	1.21	1.20
5.	18	42.7	25.2	0	42	1.01	0.97	2.20	1.93
6.	19	42.7	26.2	0	40	2.10	2.37	5.20	5.22
7.	20	42.2	25.9	0	44	4.06	5.10	6.13	7.23
8.	21	42.1	26.8	0	45	10.67	9.57	9.12	8.37
9.	22	40	26.5	21	61	12.31	12.63	10.13	10.02
10.	23	33.1	21.7	112.8	89	25.50	27.09	17.11	16.13
11.	24	36.1	23.8	53.2	78	0	0	21.06	20.37
12.	25	34	22.8	112.5	84	0	0	25.13	24.87
13.	26	31.8	22.3	26.3	84	0	0	27.47	27.08
14.	27	31.8	22.4	48.3	87	0	0	29.15	28.49
15.	28	28.9	21.9	39.9	92	0	0	30.11	30.17
16.	29	29.6	21.8	103.4	88	0	0	32.16	31.98
17.	30	29.9	21.9	2.2	83	0	0	35.37	35.22
18.	31	32.8	21.7	0	79	0	0	37.16	37.17
19.	32	30.5	22	7.2	84	0	0	38.18	37.97
20.	33	28.3	21.5	148.4	90	0	0	39.50	40.12
21.	34	28.7	20.5	110.2	91	0	0	42.17	43.37
22.	35	29.7	20.8	8.8	84	0	0	47.18	46.17
23.	36	30.9	20.2	2.4	83	0	0	51.12	52.55
24.	37	33	20.7	0	83	0	0	54.17	55.12

SMW: Standard Metrological Week, PDI : Per cent Disease Incidence, PDS: Per cent Disease Severity

The data on meteorological factors and disease development studies revealed that the disease first appeared in 14th standard meteorological week on leaf

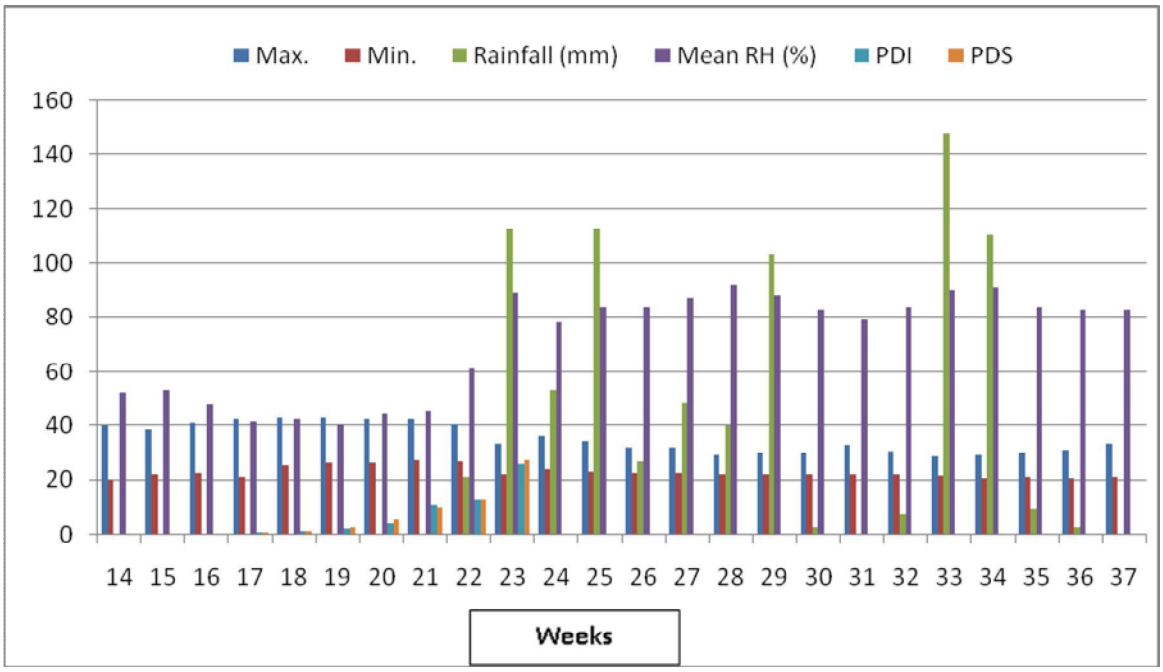


Fig. 4.10. Development of disease in relation to weather parameters under field conditions during 2018 (on fruits)

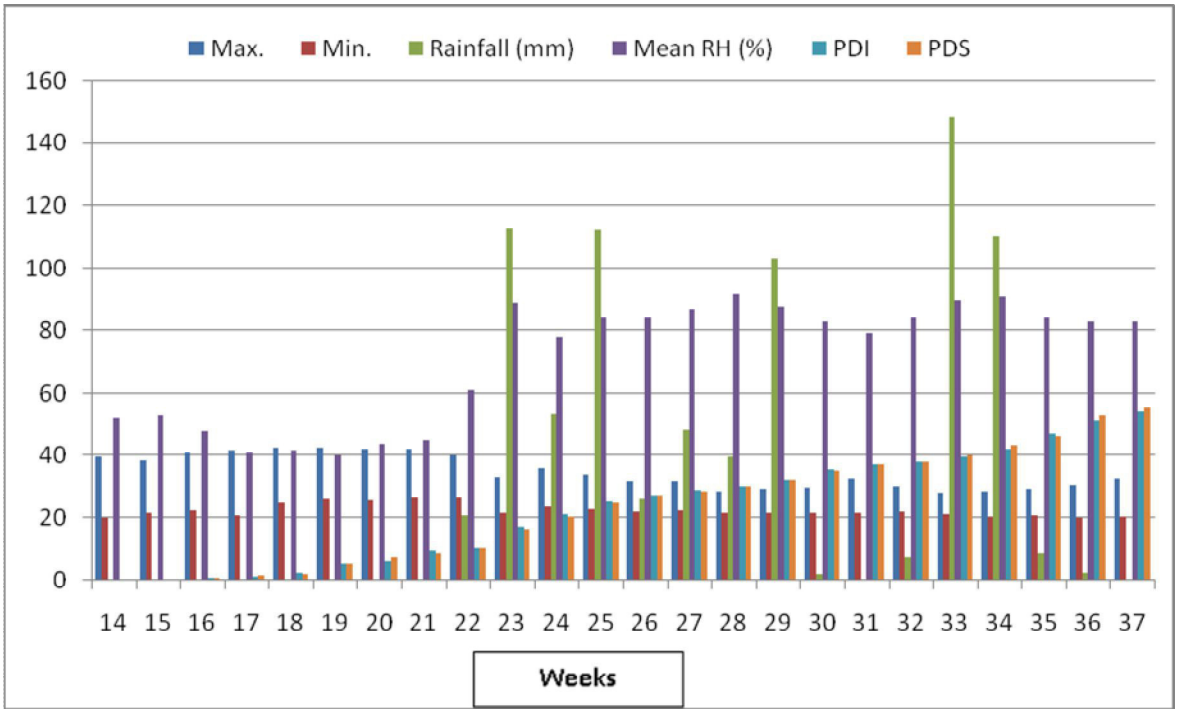


Fig. 4.11. Development of disease in relation to weather parameters under field conditions during 2018 (on leaves)

with the prevalence of maximum and minimum temperature, relative humidity and annual rainfall upto the extent of 39.9 and 19.9 ^{0}C, 52 per cent and no rainfall, respectively and 16th standard meteorological week on fruit with the prevalence of maximum and minimum temperature, relative humidity and annual rainfall of 40.9 and 22.4 ^{0}C, 48 per cent and no rainfall, respectively. Per cent disease incidence and severity of bacterial leaf spot on leaf progressed at upto 18th standard meteorological week and thereafter, a rapid progress was recorded. On the fruits the per cent disease incidence and severity of bacterial leaf spot of mango progressed at slow rate upto 16th standard meteorological week and thereafter, a rapid progress was recorded upto the harvesting of the fruit.

4.8.4. Apparent infection rate and area under disease progress curve in the year 2018-19.

Table 4.20: Apparent infection rate and area under disease progress curve in the year 2018-19

Sr. No.	SMW	Fruit			Leaf		
		PDS	r	AUDPC	PDS	r	AUDPC
1.	14	0	-	-	0.12	-	1.37
2.	15	0	-	-	0.27	-	1.68
3.	16	0.11	-	1.52	0.59	-	2.21
4.	17	0.71	-	2.61	1.20	0.091	3.97
5.	18	0.97	-	3.63	1.93	0.098	6.56
6.	19	2.37	0.187	13.53	5.22	0.187	35.63
7.	20	5.10	0.289	37.61	7.23	0.133	107.56
8.	21	9.57	0.128	107.36	8.37	0.097	126.51
9.	22	12.63	0.093	146.66	10.02	0.086	136.61
10.	23	27.09	0.071	187.83	16.13	0.078	157.76
11.	24	0	-	-	20.37	0.071	166.21
12.	25	0	-	-	24.87	0.062	179.11
13.	26	0	-	-	27.08	0.068	189.87
14.	27	0	-	-	28.49	0.052	195.39
15.	28	0	-	-	30.17	0.043	210.61
16.	29	0	-	-	31.98	0.041	235.86
17.	30	0	-	-	35.22	0.062	260.11
18.	31	0	-	-	37.17	0.069	285.21
19.	32	0	-	-	37.97	0.076	310.61
20.	33	0	-	-	40.12	0.081	320.81
21.	34	0	-	-	43.37	0.086	344.31
22.	35	0	-	-	46.17	0.051	368.71
23.	36	0	-	-	52.55	0.043	390.66
24.	37	0	-	-	55.12	0.041	411.26
Total				**500.75**			**4448.59**

The apparent infection rate (r) is an estimate of rate of progress of a disease, based on proportional measures of the extent of infection at different time intervals. The apparent infection rate (r) or the rate of progress of severity of bacterial leaf spot of mango in the year 2018-19 (Table 4.20 and Fig. 4.12 & 4.13) progressed at a proportion of 0.187 on fruits per week interval of time under field condition and the rate of progresses of severity of bacterial leaf spot progressed at a proportion of 0.071 per week. The apparent infection rate (r) on incidence of bacterial leaf spot of mango on leaves in the year 2018-19 progresses at a proportion of 0.091 per week and the apparent infection rate on severity of bacterial leaf spot progresses at 0.041 weeks time interval on leaves. The area under disease progress curve (AUDPC) for the severity of bacterial leaf spot of mango on fruits under field conditions in the year 2018-19 was 500.75 and the severity of bacterial leaf spot of mango on leaves was 4448.59.

4.8.5. Progress of disease under field conditions in the year 2019-20

The data on meteorological factors and disease development studies for the year 2019-20 revealed that (Table 4.21 and Fig. 4.14 & 4.15), the disease first appeared in 15th standard meteorological week on leaf with the prevalence of maximum and minimum temperature, relative humidity and annual rainfall upto the extent of 42 and 22.2 ^{0}C, 38 per cent and 1.6 mm, respectively and 16th standard meteorological week on fruit with the prevalence of maximum and minimum temperature, relative humidity and annual rainfall of 39.3 and 21.4 ^{0}C, 40 per cent and no rainfall, respectively. Per cent disease incidence and severity of bacterial leaf spot on leaf progressed at upto 18th standard meteorological week and thereafter, a rapid progress was recorded.

Table 4.21: Development of disease in relation to weather parameters under field conditions during, 2019-20

Sr. No.	SMW	Mean Temp (^{0}C)		Rainfall (mm)	Mean RH (%)	Fruit		Leaves	
		Max.	Min.			PDI	PDS	PDI	PDS
1.	14	40.9	21.3	0.0	40	0	0	0	0

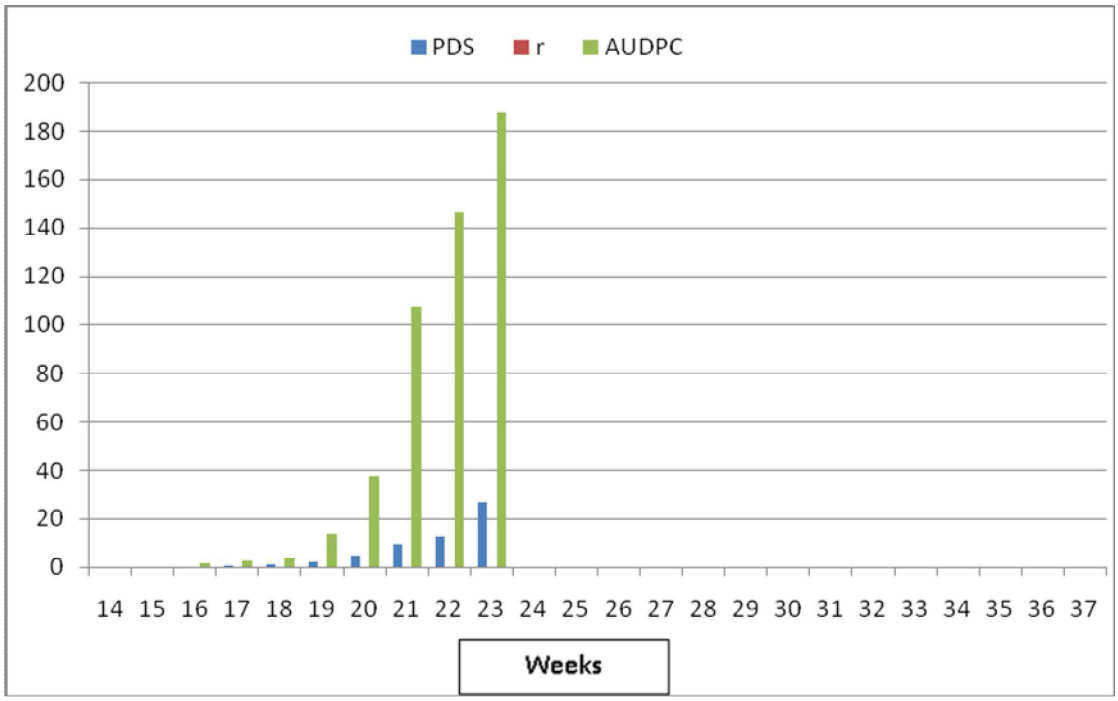


Fig. 4.12. Apparent infection rate and AUDPC (on fruit) in the year 2018

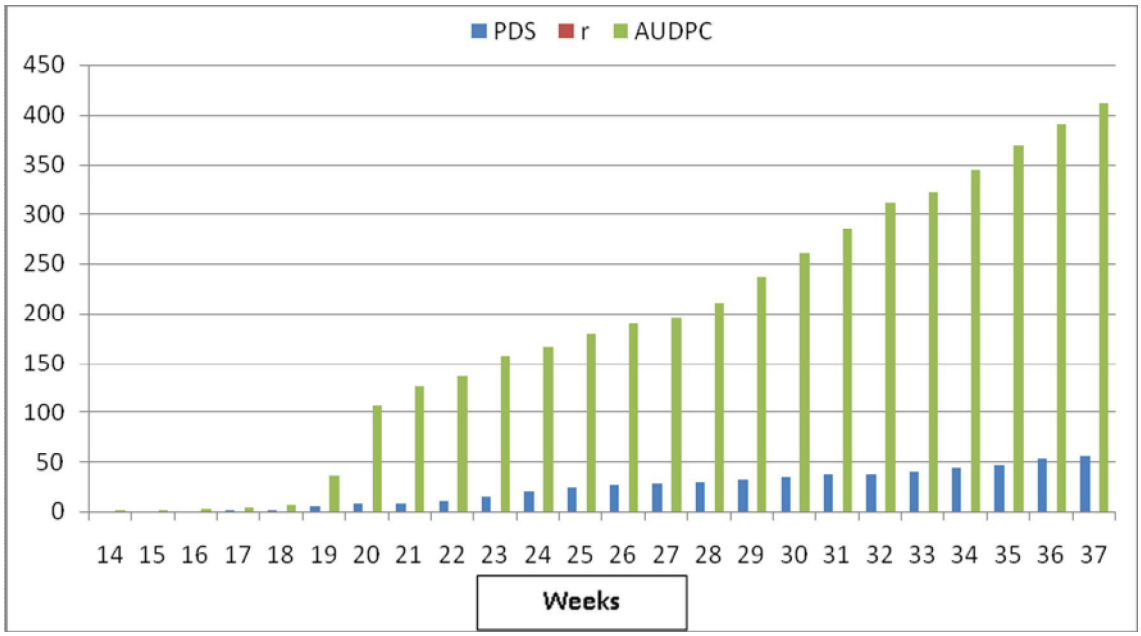


Fig. 4.13. Apparent infection rate and AUDPC (on leaves) in the year 2018

2.	15	42.0	22.2	1.6	38	0	0	0.23	0.31
3.	16	39.3	21.4	0.0	40	0.21	0.19	0.29	0.32
4.	17	43.9	24.5	0.0	36	0.29	0.20	0.32	0.41
5.	18	41.6	24.2	0.0	39	0.29	0.21	0.41	0.46
6.	19	41.8	26.1	0.0	35	3.39	4.40	1.21	1.32
7.	20	42.4	24.8	0.0	41	10.10	11.30	1.67	1.62
8.	21	44.1	27.1	0.0	38	12.23	11.69	1.77	1.92
9.	22	43.9	28.7	0.0	36	15.63	16.23	5.23	6.21
10.	23	40.9	24.8	33.7	76	0	0	6.21	6.97
11.	24	39.3	25.2	0.0	62	0	0	9.28	10.18
12.	25	35.3	24.7	10.5	69	0	0	16.21	15.11
13.	26	33.2	22.7	46.9	85	0	0	18.98	19.10
14.	27	33.2	23.1	10.6	76	0	0	22.93	25.61
15.	28	33.5	22.6	34.2	83	0	0	25.42	26.11
16.	29	34.2	22.9	11.2	79	0	0	35.13	33.11
17.	30	30.6	22.6	64.3	81	0	0	39.12	39.62
18.	31	28.1	21.8	85.4	92	0	0	42.40	42.11
19.	32	30.5	22.0	62.2	89	0	0	42.55	43.23
20.	33	32.3	21.5	9.7	80	0	0	43.07	43.66
21.	34	32.2	22.0	1.2	80	0	0	53.22	58.67
22.	35	31.2	21.5	78.0	88	0	0	55.11	60.12
23.	36	30.1	21.6	13.2	83	0	0	61.22	72.10
24.	37	30.0	21.2	86.4	88	0	0	65.27	73.11

SMW: Standard Metrological Week, PDI : Per cent Disease Incidence, PDS: Per cent Disease Severity

4.8.6. Apparent infection rate and area under disease progress curve in the year 2019-20

The apparent infection rate (r) or the rate of progress of severity of bacterial leaf spot of mango in the year 2019-20 (Table 4.22 and Fig. 4.16 & 4.17) progressed at a proportion of 0.269 on fruits per week interval of time under field condition and the rate of progresses of severity of bacterial leaf spot progressed at a proportion of 0.070 per week. The apparent infection rate (r) on incidence of bacterial leaf spot of mango on leaves in the year 2019-20 progresses at a proportion of 0.089 per week and the apparent infection rate on severity of bacterial leaf spot progresses at 0.049 weeks time interval on leaves. The area under disease progress curve (AUDPC)

for the severity of bacterial leaf spot of mango on fruits under field conditions in the year 2019-20 was 356.96 and the severity of bacterial leaf spot of mango on leaves was 4036.12.

Table 4.22: Apparent infection rate and area under disease progress curve in the year 2019-20

Sr. No.	SMW	Fruit			Leaf		
		PDS	r	AUDPC	PDS	r	AUDPC
1.	14	0	-	-	0	-	-
2.	15	0	-	-	0.31	-	1.83
3.	16	0.19	-	1.67	0.32	-	1.97
4.	17	0.20	-	1.72	0.41	-	2.19
5.	18	0.21	-	1.76	0.46	-	2.23
6.	19	4.40	0.269	19.79	1.32	0.089	6.25
7.	20	11.30	0.283	47.37	1.62	0.093	17.33
8.	21	11.69	0.127	107.32	1.92	0.097	19.12
9.	22	16.23	0.070	177.33	6.21	0.192	22.27
10.	23	0	-	-	6.97	0.194	23.63
11.	24	0	-	-	10.18	0.154	51.32
12.	25	0	-	-	15.11	0.034	91.06
13.	26	0	-	-	19.10	0.071	133.86
14.	27	0	-	-	25.61	0.062	160.33
15.	28	0	-	-	26.11	0.060	170.31
16.	29	0	-	-	33.11	0.052	190.29
17.	30	0	-	-	39.62	0.043	240.22
18.	31	0	-	-	42.11	0.061	295.51
19.	32	0	-	-	43.23	0.068	320.69
20.	33	0	-	-	43.66	0.082	367.83
21.	34	0	-	-	58.67	0.056	420.71
22.	35	0	-	-	60.12	0.052	480.61
23.	36	0	-	-	72.10	0.051	496.23
24.	37	0	-	-	73.11	0.049	520.33
Total				**356.96**			**4036.12**

4.8.7. Correlation with weather factors in the year 2018-19

4.8.7.1. Influence of maximum temperature on disease progress

Generally, the maximum temperature had negative influence on the disease progress / decline as observed in many cases. But the correlation did not followed the similar trend on fruit because the disease progresses with maximum temperature were positive with disease severity on fruit with correlation of 0.197. On

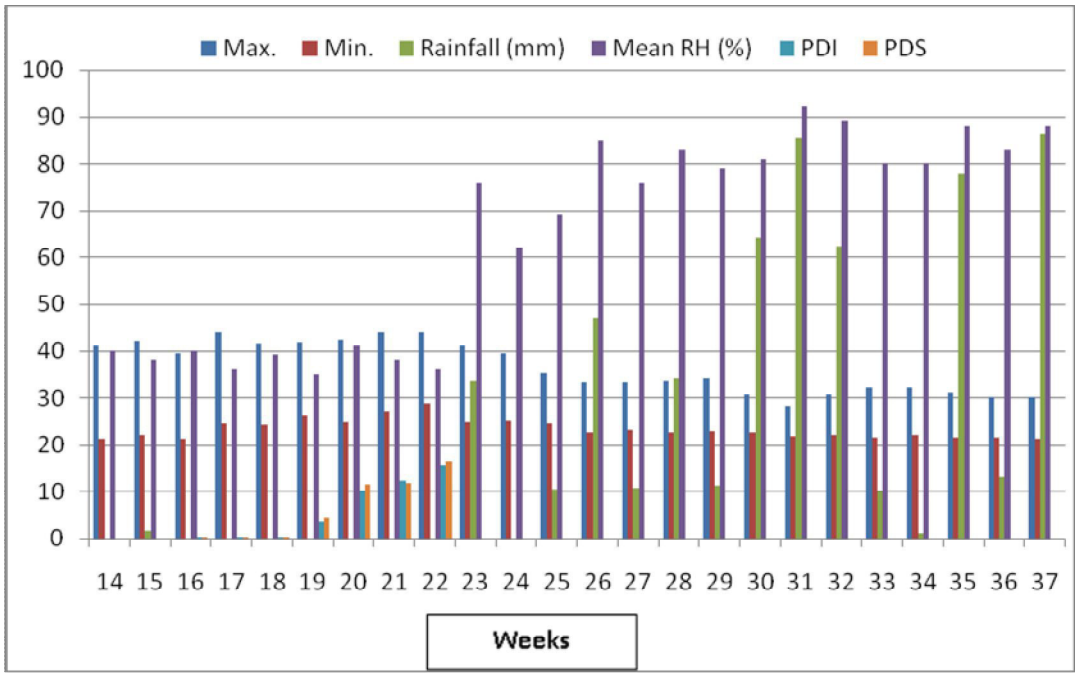


Fig. 4.14. Development of disease in relation to weather parameters under field conditions during 2019 (on fruit)

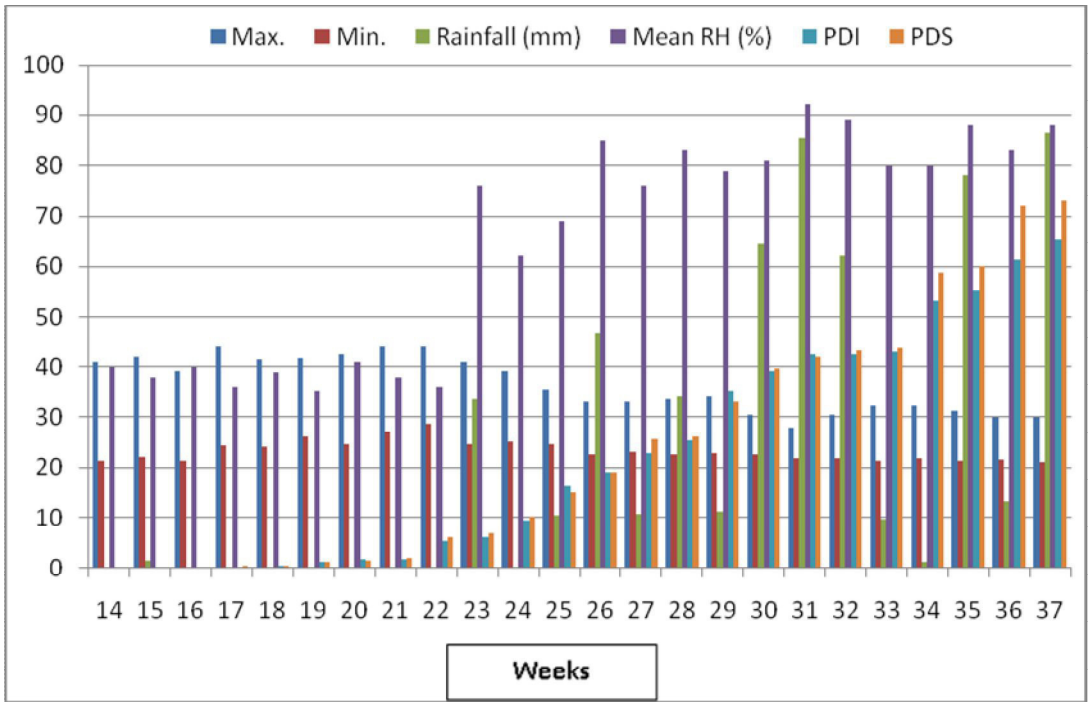


Fig. 4.15. Development of disease in relation to weather parameters under field conditions during 2019 (on leaves)

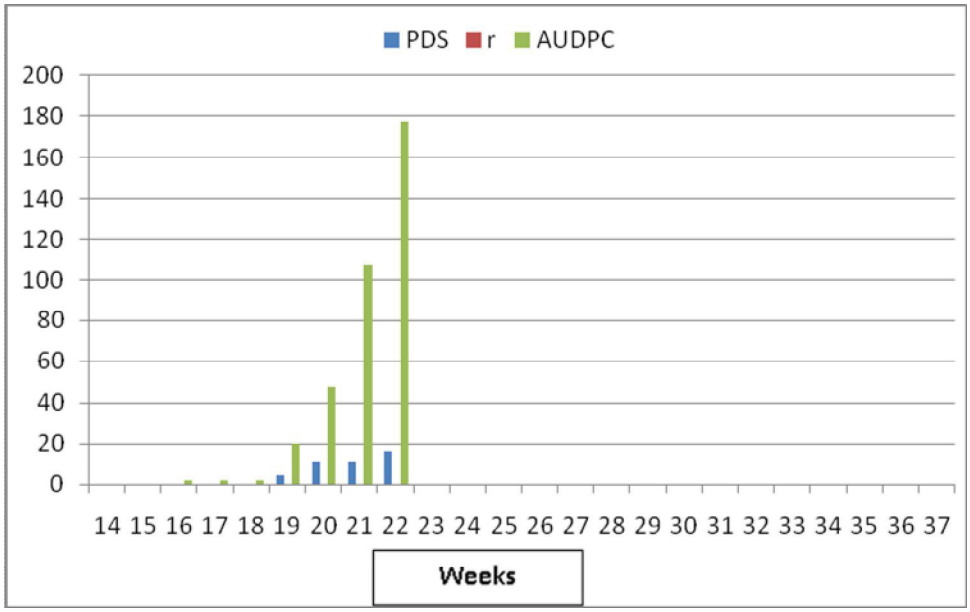


Fig. 4.16. Apparent infection rate and AUDPC (on fruit) in the year 2019

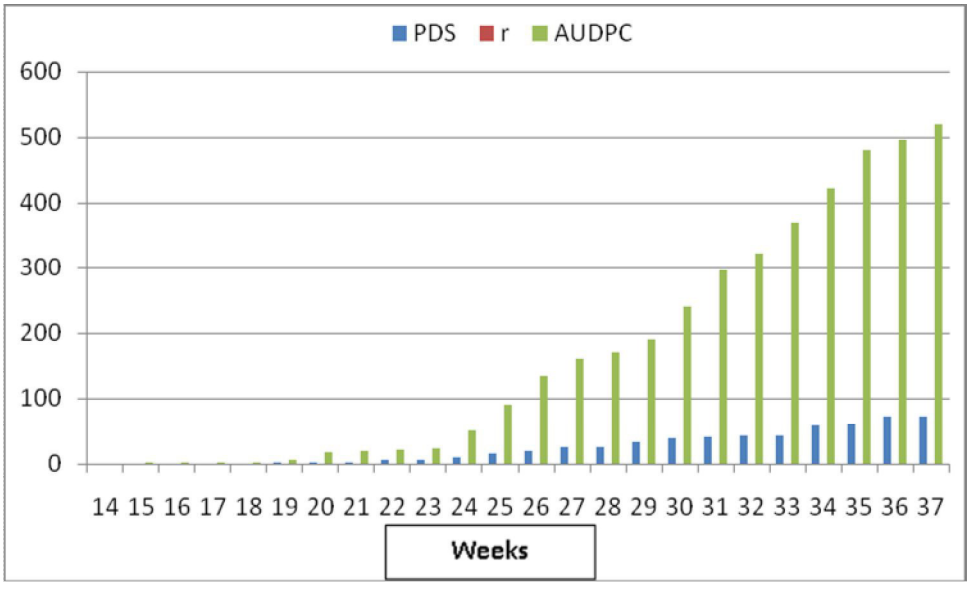


Fig. 4.17. Apparent infection rate and AUDPC (on leaves) in the year 2018

the leaf, the disease severity was negatively and significantly correlated with maximum temperature (0.865) means with increase in maximum temperature, there was significant decrease in PDS on leaves of mango.

4.8.7.2. Role of Minimum temperature in disease progress

The effect of minimum temperature on PDS of fruit was though positively correlated (0.320) but the correlation was non significant. On leaf, the disease severity of bacterial leaf spot of mango was negatively and significantly correlated with minimum temperature (-0.519) indicating that increase in minimum temperature also there was significant decrease in PDS on leaves of mango.

Table 4.23: Correlation between disease and weather variables during 2018-19

	Simple correlation 'r'			
	Max temp(^{o}C)	**Min temp(^{o}C)**	**Relative humidity (%)**	**Rainfall (mm)**
PDS on fruit	0.197(NS)	0.320(NS)	-0.055(NS)	0.205(NS)
PDS on Leaves	-0.865***	-0.519**	0.832***	0.281(NS)

*Significant at 1% level, **Significant at 5% level, ***Significant at 10% level NS: Non Significant

4.8.7.3. Effect of relative humidity on disease progress

The effect of relative humidity had negative correlation with the disease severity on fruit and had significant positive correlation with disease severity on leaves exhibiting that the increase in relative humidity, the PDS on leaves non significantly increased (0.832) but it was not so in correlation of relative humidity with that of the PDS on the fruit (-0.055).

4.8.7.4. Effect of rainfall on disease progress

The effect of rainfall also had a positive correlation with disease severity of both fruit and leaves. The rate of infection of disease with respect to disease severity on fruit was 0.205 and the rate of infection of disease with respect to disease severity on leaves was 0.281 but the correlation was non significant.

4.8.8. Coefficient of multiple determinations in the year 2018-19

The effect of weather variables was found significantly favorable for bacterial leaf spot development and spread the disease. 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 0.32 to 0.80 indicating minimum of 32 per cent (PDS on fruit) to the maximum of 80 per cent (PDS on leaf) forecastability during the year 2018-19.

Table 4.24: Multiple regression models for bacterial leaf spot of mango in relation to weather factors during 2018-19

	Coefficient of multiple determination(R^2)	Regression models
PDS on fruit	0.320	Y= -104.55+1.831(X_1)+0.454 (X_2)+0.027(X_3)+0.447(X_4)
PDS on Leaves	0.80	Y= 83.722-2.585(X_1)+0.611 (X_2-0.106(X_3)+0.280(X_4)

Y= Disease incidence/disease severity, X1= Maximum temperature, X2= Minimum temperature, X3= Relative humidity, X4= Rainfall

4.8.9 Correlation with weather factors in the year 2019-20

In the year of 2019 the role of weather variables were assessed to know the progress of disease under field conditions.

4.8.9.1. Influence of maximum temperature on disease progress

The role of maximum temperature had positive influence on the disease progress. The correlation followed the similar trend in which the disease progressed in the previous year *i.e* 2018-19 with the maximum temperature, but it was significantly positive (0.545) on fruit. On the leaf, the disease severity and maximum temperature had significant negative correlation (-0.881). Thus the role of maximum temperature is significant in disease severity of fruit but had significant negative role in disease progress on leaves (Table 4.25).

4.8.9.2. Role of minimum temperature with disease progress

The effect of minimum temperature was again positively correlated with the disease progress on fruit and negatively correlated with disease progress leaf as in previous year. The minimum temperature is significantly positively correlated 0.762 with the disease severity on fruit. On leaf, the correlation between minimum temperature and disease severity was significantly negative (-0.587) at 5 per cent level.

Table 4.25: Correlation between disease and weather variables during 2019-20

	Simple correlation 'r'			
	Max temp(^{o}C)	**Min temp(^{o}C)**	**Relative humidity (%)**	**Rainfall (mm)**
PDS on fruit	0.545**	0.762***	-0.518**	-0.316(NS)
PDS on Leaves	-0.881***	-0.587**	0.822***	0.627**

*Significant at 1% level, **Significant at 5% level, ***Significant at 10% level NS: Non Significant

4.8.9.3. Effect of relative humidity on disease progress

The effect of relative humidity had negative correlation with the disease severity of the fruit and positive correlation with the disease severity of leaf which was highly significant for the disease progress in the field condition. The correlation of relative humidity with respect to disease severity on fruit was negative (-0.518) and significant at 5 per cent level. The correlation of relative humidity with respect to disease severity on leaf was positive and significant (0.822) at 10 per cent level.

4.8.9.4. Effect of rainfall on disease progress

The effect of rainfall had negative correlation with the disease severity of fruit, but had significant positive correlation with that of disease severity on leaf in the field condition. The correlation of occurrence of rainfall with respect to disease severity on fruit was -0.316. The correlation of occurrence of rainfall with respect to disease severity on leaf was 0.627 which was significant at 5 per cent level.

4.8.10. Coefficient of multiple determinations in the year 2019-20

Table 4.26: Multiple regression models for bacterial leaf spot of mango in relation to weather factors during 2019

	Coefficient of multiple determination(R^2)	Regression models
PDS on fruit	0.64	Y= -16.685-0.424(X_1)+1.790 (X_2)+0.024(X_3)-0.131(X_4)
PDS on Leaves	0.78	Y= 162.142-4.176(X_1)+0.590 (X_2)-0.013(X_3)+0.012(X_4)

Y= Disease incidence/disease severity, X1= Maximum temperature, X2= Minimum temperature, X3= Relative humidity, X4= Rainfall

The effect of weather variables during the year 2019-20 was found significantly favorable for bacterial leaf spot development and spread the disease. 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 0.64 to 0.78 indicating minimum of 64 per cent (PDS on fruit) to the maximum of 78 per cent (PDS on leaf) forecastability during the year 2019-20.

4.8.11. Correlation with weather factors of two consecutive years *i.e* 2018-19 and 2019-20

The pooled data in Table 4.27 for the two consecutive years 2018-19 and 2019-20 were assessed to know the epidemiological factors for the progress of disease in relation to weather factors under field conditions.

Table 4.27: Correlation with weather factors of two consecutive years *i.e* 2018-19 and 2019-20

	Simple correlation 'r'			
	Max temp (^{0}C)	**Min temp (^{0}C)**	**RH (%)**	**Rainfall (mm)**
PDS on fruit	0.324*	0.471***	0.050	-0.240
PDS on leaves	-0.854***	-0.536***	0.407**	0.809***

*Significant at 1% level, **Significant at 5% level, ***Significant at 10% level NS: Non Significant

4.8.11.1. Influence of maximum temperature with disease progress

The role of maximum temperature had positive and negative influence on the disease progress of fruit and leaves respectively, recorded in the two seasons of 2018-19 and 2019-20. The correlation with disease severity observed on fruit was 0.324 which was significant at 1 per cent level. On the leaf the disease severity observed was also significantly negative with disease severity of -0.854 which was significant at 10 per cent level. Thus the maximum temperature had no role in disease progress on leaves but had role in disease progress on fruits under field conditions of two consecutive years.

4.8.11.2. Role of minimum temperature with disease progress

The effect of minimum temperature was positively correlated with disease progress as the rate of infection was significantly correlated with the disease. The correlation between minimum temperature and disease severity on fruit was 0.471 which was significant at 10 per cent level. On leaf the correlation between minimum temperature and disease severity of bacterial leaf spot of mango was -0.536 which was significant at 10 per cent level.

4.8.11.3. Effect of relative humidity with disease progress

The effect of relative humidity also had a positive correlation with disease severity of fruit and negative correlation of leaf which was significant for disease progress in the field condition. The correlation between relative humidity with respect to disease severity on fruit was 0.050. The correlation between relative humidity and disease severity on leaf was 0.470 which was significant at 5 per cent level.

4.8.11.4. Effect of rainfall with disease progress

The effect of rainfall had negative correlation of fruit and positive correlation of leaf with disease severity which was significant for disease progress in

the field conditions. The correlation between rainfall and disease severity on fruit was -0.240. The correlation between rainfall and disease severity of leaf was 0.809 which was significant at 10 per cent level.

4.8.12. Coefficient of multiple determinations in the year 2018 and 2019

Combined effect of weather variables was found significantly favorable for bacterial leaf spot development and spread of the disease. Step-wise regression models developed between the disease development and weather variables (Table 4.21) revealed the strong significant relationship between the combined effect of weather factors and disease development with values (R^2) ranged between 0.29 to 0.75 indicating minimum of 39 per cent (PDS on fruit) to the maximum of 75 per cent (PDS on leaf) forecastability of pooled data.

Table 4.28: Multiple regression models for bacterial leaf spot of mango in relation to weather factors during 2018-19 and 2019-20

	Coefficient of multiple determination(R^2)	Regression models
PDS on fruit	0.29	$Y=-38.732+0.380(X_1)+1.021(X_2)+0.040(X_3)+0.040(X_4)$
PDS on Leaves	0.75	$Y=128.964-3.608(X_1)+0.811(X_2)\ 0.092(X_3)+0.113(X_4)$

Y= Disease incidence/disease severity, X1= Maximum temperature, X2= Minimum temperature, X3= Relative humidity, X4= Rainfall

Manicom (1986) stated that the bacterial black spot of mango caused by *X. campestris* pv. *mangiferaeindicae* found to be essentially a wound pathogen of leaves. Maximum infection occurring at a temperature regime of $22/26^0$C (night/day). The major weather factor affecting fruit infection was rainfall which showed a significant correlation (r=0-77) with levels of infection after allowing for an approximate two week latent period. The results are in agreement with the work of Shukla and Gupta (2005) who obtained a positive correlation between relative humidity, temperature, soil moisture and rainfall with incidence and severity of bacterial leaf spot of tomato. 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. Shukla and Gupta (2005) studied

epidemiological aspects of bacterial leaf spot of tomato, where multiple regression equation depicted strong significant positive relationship between weather variables (temperature, relative humidity, rainfall and soil moisture) and bacterial leaf spot severity in tomato.

4.9. Disease management strategies

4.9.1. *In vitro* efficacy of antibiotics against *X. campestris* pv. *mangiferaeindicae*

Total seven antibiotics (each @ recommended field dosage, 50 % of recommended and 125 % of recommended), were evaluated alone and with combination of fungicides *in vitro* by applying inhibition zone assay technique. The results obtained on inhibition zone of test bacterium are presented (Table 4.29).

Table 4.29: *In vitro* efficacy of antibiotics against *X. campestris* pv. *mangiferaeindicae*

Tr. No.	Treatments	Mean Inhibition Zone (mm)*			Av. Inhibition Zone (mm)
		50% RD	100% RD	125% RD	
T_1	Streptomycin sulphate 90%w/w + Tetracycline hydrochloride 10% (Streptocycline)	12.06 (20.32)	16.00 (23.58)	17.84 (23.57)	15.30
T_2	2-Bromo 2-Nitro propane 1,3-diol (Bacterinashak)	9.98 (18.41)	12.23 (20.74)	13.07 (21.19)	11.76
T_3	Kasugamycin (Biomycin)	9.55 (18.00)	10.45 (18.86)	12.40 (20.61)	10.80
T_4	Streptomycin sulphate 9% w/w + Tetracycline hydrochloride 1% w/w (Plantomycin)	11.15 (19.51)	13.90 (21.89)	15.45 (20.66)	13.50
T_5	Kasugamycin 5%+ Copper oxycloride 45% WP(Conika)	9.85 (18.29)	12.78 (20.95)	13.77 (21.78)	12.15
T_6	Bacterinol	9.12 (17.58)	12.25 (20.48)	14.04 (22.05)	11.88
T_7	Bacterinol + Copper oxychloride	10.03 (18.46)	12.11 (20.64)	15.84 (23.45)	12.66
T_8	Control (untreated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	S.E. ±	**0.25**	**0.14**	**0.22**	--
	C.D. (P=0.01)	**0.74**	**0.42**	**0.64**	--

*Mean of three replications
Figures in parentheses are arcsine transformed values

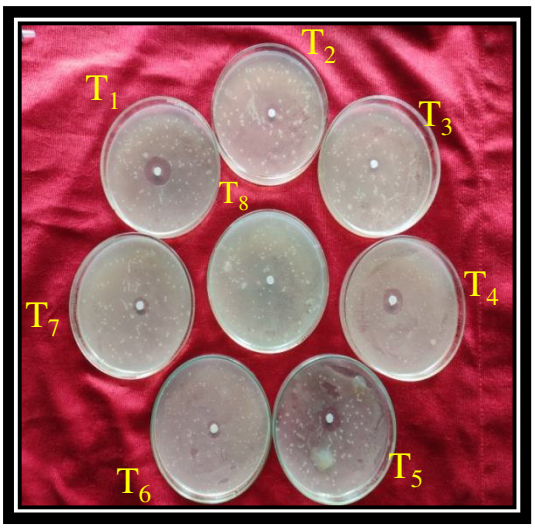
Results (Table 4.29, Plate 4.15 and Fig. 4.18) indicated that the antibiotics tested at various concentrations (each @ RD, 50% RD and 125% RD) significantly inhibited growth of *X. campestris* pv.*mangiferaeindicae*, over control (untreated).

At 50 per cent of recommended dose, bacterial mean inhibition zone was ranged from 9.12 mm (Bacterinol) to 12.06 mm (Streptocycline). However, it was significantly highest with streptocycline (12.06 mm), followed by Plantomycin (11.15 mm) but were at par, Bacterinol + Copper oxychlorode (10.03 mm), Bacterinashak (9.98 mm), Conika (9.85mm), Biomycin (9.55 mm). Whereas, significantly least inhibition zone was found with Bacterinol (9.12 mm), but were at par with the treatments *viz.,* Bacrterinashak, Conika and Biomycin.

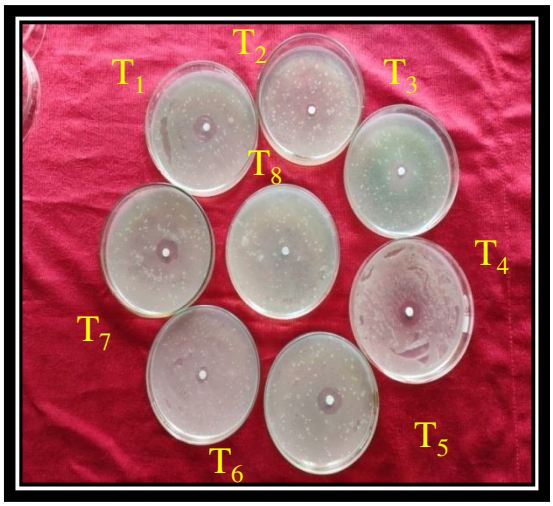
At 100 per cent of recommended dose, bacterial mean inhibition zone was ranged from 10.45 mm (Kasugamycin) to 16.00 mm (Streptocycline). However, it was significantly highest with Streptocycline (16.00 mm), followed by Plantomycin (13.90 mm), Bacterinol + Copper oxychloride (12.91 mm), but both the treatments were at par with each other. Whereas, significantly least inhibition zone was found with Bacterinol (10.45 mm) while, the treatments viz., Conika (12.78 mm), Bacterinol (12.25 mm), Bacterinashak (12.23 mm) were at par with each other.

At 125 per cent of recommended dose, bacterial mean inhibition zone was ranged from 12.40 mm (Kasugamycin) to 17.84 mm (Streptocycline). However, it was significantly highest with Streptocycline (17.84 mm), followed by Plantomycin (15.45 mm), Bacterinol + Copper oxychloride (15.45 mm), Bacterinol (14.04 mm), Conika (13.77 mm) but were at par with each other, Bacterinashak (13.07 mm). Whereas, significantly least inhibition zone was found with Biomycin (12.40 mm).

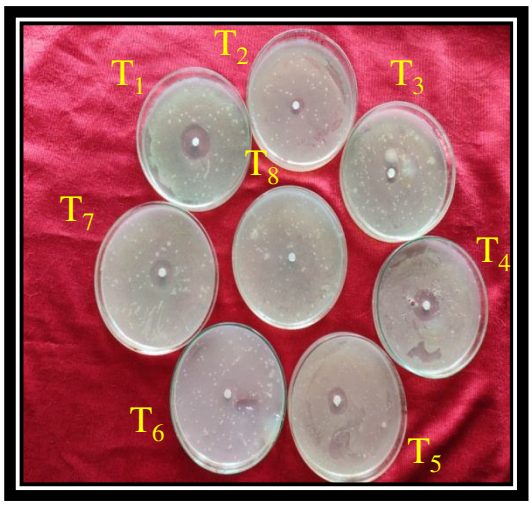
Average inhibition zone was ranged from 10.80 mm (Biomycin) to 15.30 mm (Streptocycline). However, it was significantly highest with 15.30 mm (Streptocycline), followed by Plantomycin (13.50 mm) and Bacterinol + Copper oxychlorode (12.66 mm), Conika (12.15 mm) and Bacterinol (11.88 mm): whereas, significantly least inhibition zone was found with Biomycin (10.80 mm).



50% RD



100% RD



125% RD

Tr. No.	Treatments
T_1	Streptomycin sulphate 90%w/w + Tetracycline hydrochloride 10% (Streptocycline)
T_2	2-Bromo 2-Nitro propane 1,3-diol (Bacterinashak)
T_3	Kasugamycin (Biomycin)
T_4	Streptomycin sulphate 9% w/w + Tetracycline hydrochloride 1% w/w (Plantomycin)

Tr. No.	Treatments
T_5	Kasugamycin 5%+ Copper oxycloride 45% WP(Conika)
T_6	Bacterinol
T_7	Bacterinol + Copper oxychloride
T_8	Control (untreated)

Plate 4.15. *In vitro* efficacy antibiotics against *X. campestris* pv. *mangiferaeindicae*

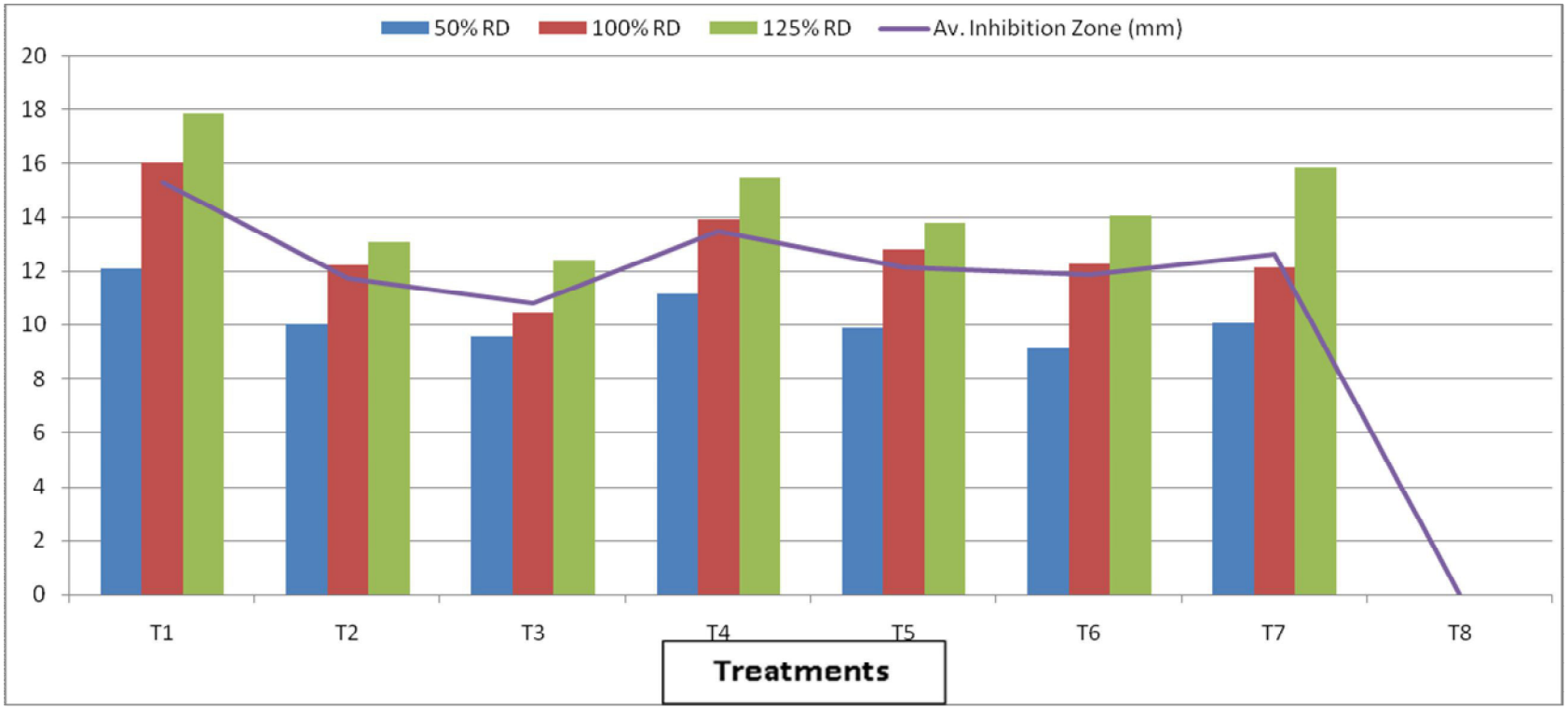


Fig. 4.18. ***In vitro*** **efficacy of antibiotics against** ***X. campestris*** **pv.** ***mangiferaeindicae***

T_1	Streptomycin sulphate 90%w/w + Tetracycline hydrochloride 10% (Streptocycline)	T_5	Kasugamycin 5%+ Copper oxycloride 45% WP (Conika)
T_2	2-Bromo 2-Nitro propane 1,3-diol (Bacterinashak)	T_6	Bacterinol
T_3	Kasugamycin (Biomycin)	T_7	Bacterinol + Copper oxychloride
T_4	Streptomycin sulphate 9% w/w + Tetracycline hydrochloride 1% w/w (Plantomycin)	T_8	Control (untreated)

Similar findings were recorded earlier by many workers. Venugopal, (1983); Bashoff, (2000); Hasan, (2005); Thind and Singh, (2015). Chirame and Shinde (1993) evaluated *in vitro* efficacy of some antibiotics against *X. campestris* pv. *citri* and reported highest zone of inhibition with streptocycline @ 500 ppm and it was least significantly with aureofungin @ 10 ppm. Sheikh *et al.,* (1995) reported that among 10 toxicants tested against *X. campestris* pv. *mangiferaeindicae,* streptomycin at 1 % was the most inhibitory. Jambenal *et al.,* (2011) tested *in vitro* efficacy of different chemicals against *X. campestris* pv. *viticola* causing bacterial leaf spot of grape. Where the Streptocycline 500 ppm + Copper oxychlorite 2000 ppm produced maximum inhibition zone (24.97 mm), followed by streptocycline @ 500 ppm (22.40 mm). Negi and kumar (2015) evaluated total seven antibiotics against *X. axonopodis* pv. *citri.* Results showed that, all the seven antibiotics tested were significantly effective. Among these, at 2000 ppm concentration, Chloramphenicol and Streptocycline showed maximum zone of inhibition *i.e* 2.83 cm and 2.75 cm respectively, followed by Tetracycline (2.73 cm) and Streptomycin (2.45 cm), while penicillin was found to be least effective at 2000 ppm. Bandi (2019) studied in vitro evaluation of antibiotics, chemicals and their combinations against *X. campestris* pv. *mangiferaeindicae*, indicated that streptocycline (22.40 mm) followed by carbendazim + streptocycline (21.96 mm) were highly effective in recording maximum mean inhibition zone. Individual concentration of the treatments tested *in vitro* revealed that, streptocycline 500 ppm (27.6 mm) followed by streptocycline + copper sulphate 250 + 2000 ppm (25.3 mm) were found superior showing maximum inhibition zone and inhibited growth of the pathogen to the extent of 30.66 and 28.11 per cent respectively.

4.9.2. *In vitro* efficacy of fungicides against *X. campestris* pv. *mangiferaeindicae*

Total seven fungicides (each @ recommended field dosage, 50 % of recommended and 125 % of recommended), were evaluated in vitro by using inhibition zone assay technique. The results obtained on inhibition zone of the test bacterium are presented (Table 4.30).

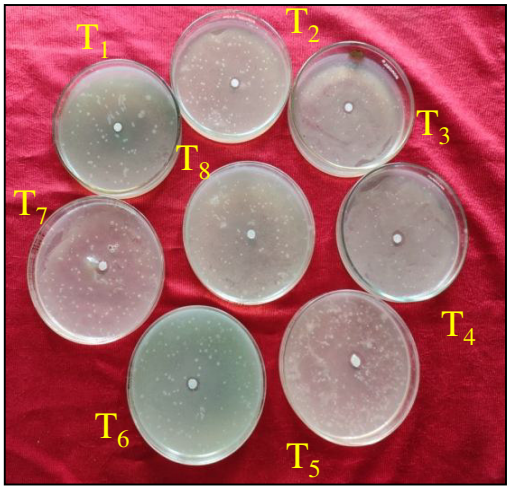
Table 4.30: *In vitro* efficacy of fungicides against *X. campestris* pv. *mangiferaeindicae*

Tr. No.	Treatments	Mean Inhibition Zone (mm)* at			Av. Inhibition Zone (mm)
		50%RD	100%RD	125%RD	
T_1	Carbendazim 50% WP	6.90 (15.22)	8.60 (17.15)	10.65 (19.05)	8.75
T_2	Bordeaux mixture	8.80 (17.26)	9.80 (18.24)	10.75 (19.05)	9.75
T_3	Mancozeb 75% WP	9.92 (18.36)	12.05 (19.87)	14.14 (22.12)	11.50
T_4	Cyamoxanil 8% + Mancozeb 64% WP	9.85 (18.29)	11.82 (20.11)	13.05 (21.15)	11.95
T_5	Copper oxychloride 50% WP	10.29 (18.71)	14.00 (21.97)	15.59 (24.45)	12.98
T_6	Copper hydroxide 77% WP	7.80 (16.22)	8.70 (17.05)	12.40 (20.62)	9.60
T_7	Clorothalonil 75%WP	8.00 (16.43)	11.80 (20.09)	12.90 (21.05)	10.90
T_8	Control (untreated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	S.E. ±	**0.92**	**0.61**	**0.12**	
	C.D. (P=0.01)	**0.27**	**1.78**	**0.35**	
*Mean of three replications Fingers in parenthesis are angular transformed values					

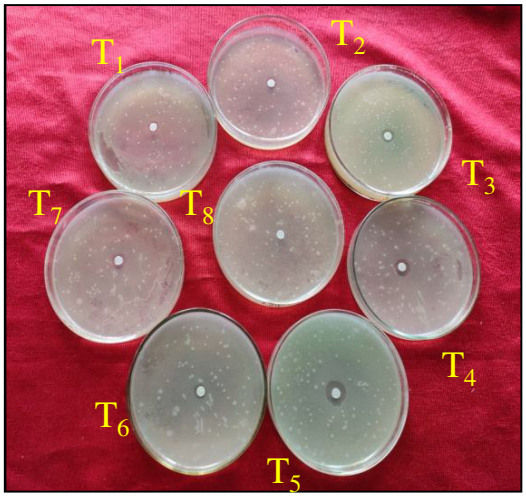
Results (Table 4.30, Plate 4.16 and Fig. 4.19) indicated that the fungicides tested at various concentrations (each @ RD, 50% RD and 125% RD) significantly inhibited growth of *X. campestris* pv. *mangiferaeindicae*, over control (untreated).

At 50 per cent of recommended dose, bacterial mean inhibition zone ranged from 6.90 mm (Carbendazim 50 % WP) to 10.29 mm (Copper oxychloride 50% WP). However, it was significantly highest with Copper oxychloride 50% WP (10.29 mm), followed by Mancozeb 75 WP (9.92 mm), Cyamoxanil 8% + Mancozeb 64% WP (9.85 mm) but were at par and Bordeaux mixture (8.80 mm); whereas, significantly least inhibition zone was found with Carbendazim 50 WP (6.90 mm).

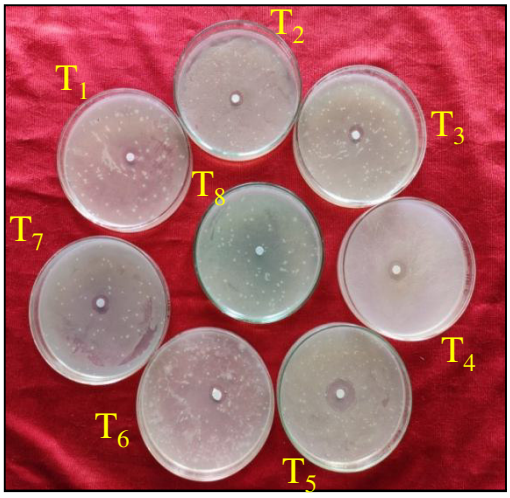
At 100 per cent of recommended dose, bacterial mean inhibition zone ranged from 8.60 mm (Carbendazim 50 % WP) to 14.00mm (Copper oxychloride 50% WP). However, it was significantly highest with Copper oxychloride 50% WP (14.00 mm), followed by Mancozeb 75 WP (12.05 mm), Cyamoxanil 8% + Mancozeb 64% WP (11.82 mm) but were at par with Chlorothalonil 75% WP (11.80 mm) and Bordeaux mixture (9.80 mm); whereas, significantly least inhibition zone was found



1500ppm



1000ppm



2000ppm

Tr. No.	Treatments
T_1	Carbendazim 50% WP
T_2	Bordeaux mixture
T_3	Mancozeb 75% WP
T_4	Cyamoxanil 8% + Mancozeb 64% WP

Tr. No.	Treatments
T_5	Copper oxychloride 50% WP
T_6	Copper hydroxide 77% WP
T_7	Clorothalonil 75%WP
T_8	Control (untreated)

Plate 4.16. *In vitro* efficacy fungicides against *X. campestris* pv. *mangiferaeindicae*

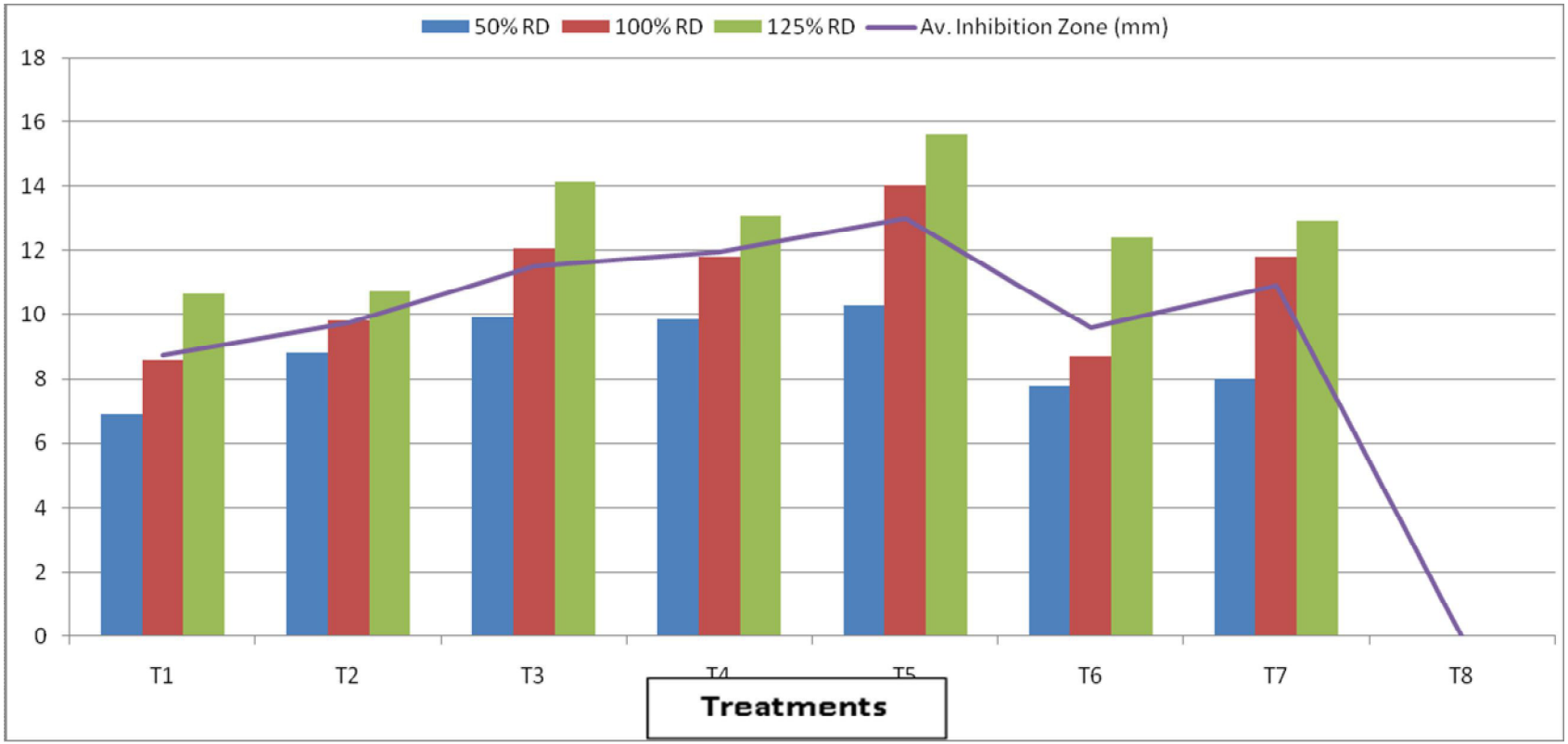


Fig. 4.19. ***In vitro*** **efficacy of fungicides against** ***X. campestris*** **pv.** ***mangiferaeindicae***

T_1	Carbendazim 50% WP	T_5	Copper oxychloride 50% WP
T_2	Bordeaux mixture	T_6	Copper hydroxide 77% WP
T_3	Mancozeb 75% WP	T_7	Clorothalonil 75%WP
T_4	Cyamoxanil 8% + Mancozeb 64% WP	T_8	Control (untreated)

with Carbendazim 50 WP (8.60 mm) but was at par with the treatment Bordeaux mixture (9.80 mm).

At 125 per cent of recommended dose, bacterial mean inhibition zone ranged from 10.65 mm (Carbendazim 50 % WP) to 15.59 mm (Copper oxychloride 50% WP). However, it was significantly highest with Copper oxychloride 50% WP (15.59 mm), followed by Mancozeb 75 WP (14.14 mm), Cyamoxanil 8% + Mancozeb 64% WP (13.05 mm),Chlorothalonil (12.90 mm), Copper hydroxide (12.40 mm); whereas, significantly least inhibition zone was found with Carbendazim 50 WP (10.65 mm) but was at par with Bordeaux mixture (10.75 mm).

Average inhibition zone ranged from 8.75 mm (Carbendazim 50% WP) to 12.98 mm (Copper oxychloride 50% WP). However, it was significantly highest with Copper oxychloride 50% WP (12.98 mm) followed by Cyamoxanil 8% + Mancozeb 75 % WP (11.95), Mancozeb 75 % WP (11.50), Chlorothalonil 75 % WP (10.90 mm), Bordeaux mixture (9.75 mm) and Copper hydroxide 77 % WP (9.60 mm); whereas, significantly least inhibition zone was found with Carbendazim 50 % WP(8.75 mm).

Similar findings were recorded earlier by many workers. Thirumalesh *et al.,* (2012) conducted experiment *in vitro* on six commercial formulates (copper sulphate, copper oxychloride, streptocycline, bacterinashak, mancozeb and bivistin) were evaluated four strains of *X. campestris* pv. *mangiferaeindicae* and reported that all the compounds inhibited bacterial growth afterhours incubation. Bandi (2019) studied eight different chemicals and their combinations against growth of *X. campestris* pv. *mangiferaeindicae* under *in vitro* condition at different level of concentration. Among the chemicals carbendazim + streptocycline (21.96 mm) found most superior followed by copper oxychloride (21.60 mm), mancozeb (19.73 mm), carbendazim (18.73 mm), mancozeb + copper oxychloride (18.53 mm). The minimum inhibition was made by copper sulphate treatment (14.20 mm). Jadhav (2019) tested fungicides at various concentrations (each @ RD, 50% RD and 125% RD) significantly inhibited the growth of *X. axonopodis* pv. *citri* over control. Average inhibition zone was significantly highest with copper oxychloride 50% WP (12.75 mm), followed by mancozeb 75% WP (11.61 mm), cyamoxanil 8% + mancozeb 64%

WP (11.16 mm), chlorothalonil 75% WP (10.61 mm); whereas, it was significantly least with carbendazim 50% WP (9.92 mm).

4.9.3. *In vitro* efficacy of essential oils against *X. campestris* pv. *mangiferaeindicae*

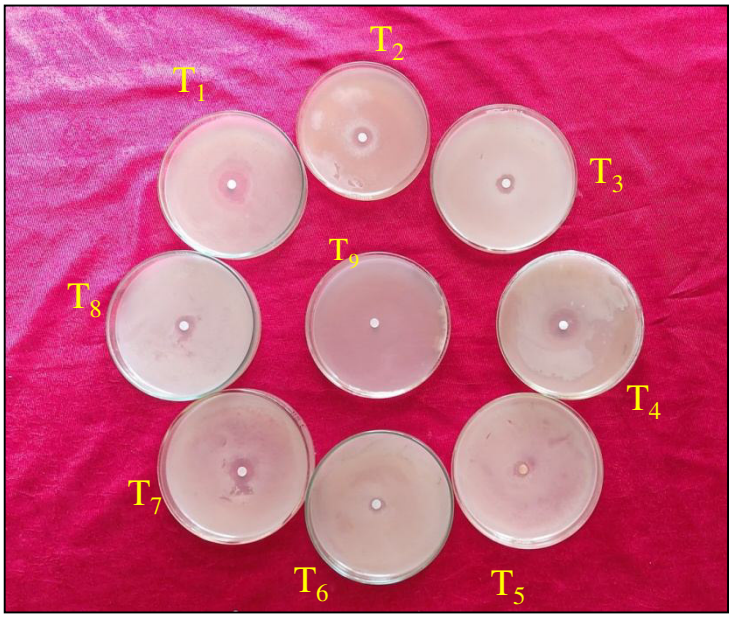
Antibacterial activity of total nine essential oils was evaluated *in vitro* (each @ 1000 and 2000 ppm) using disc diffusion. The results obtained on inhibition zone of the test bacterium are presented (Table 4.31).

Table 4.31: *In vitro* efficacy of essential oils against *X. campestris* pv. *mangiferaeindicae*

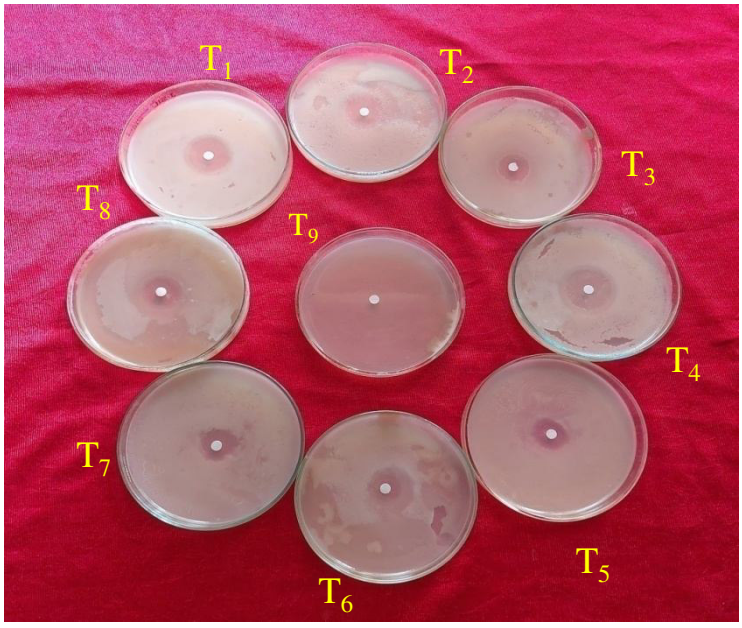
Tr. No.	Treatments	Mean Inhibition Zone (mm)*		Av. Inhibition Zone (mm)
		1000 ppm	2000 ppm	
T_1	Neem oil	11.70 (20.30)	14.50 (23.59)	13.10
T_2	Citronella oil	1050 (20.00)	12.30 (22.38)	11.40
T_3	Clove oil	9.26 (18.90)	11.26 (20.53)	10.25
T_4	Cinnamon oil	12.04 (17.91)	16.02 (19.80)	14.03
T_5	Eucalyptus oil	9.00 (17.73)	11.00 (19.62)	10.00
T_6	Mentha oil	8.70 (17.69)	11.10 (19.60)	9.90
T_7	Garlic oil	8.65 (17.45)	10.55 (19.36)	9.60
T_8	Ginger oil	9.28 (17.15)	11.28 (19.46)	10.28
T_9	Piper oil	9.52 (17.04)	11.48 (18.95)	10.50
T_{10}	Control (untreated)	0.00 (0.00)	0.00 (0.00)	0.00
	S.E.(m) ±	**0.62**	**0.56**	--
	C.D. (P=0.01)	**1.85**	**1.61**	--

*Mean of three replications
Figures in parentheses are arcsine transformed values

Results (Table 4.31, Plate 4.17 and Fig. 4.20) indicated that the essential oils tested at various concentrations (each @1000 and 2000 ppm) significantly inhibited growth of *X. campestris* pv. *mangiferaeindicae*, over control (untreated).



500ppm



1000ppm

Tr. No.	Treatments
T_1	Neem oil
T_2	Citronella oil
T_3	Clove oil
T_4	Cinnamon oil
T_5	Eucalyptus oil
T_6	Mentha oil

Tr. No.	Treatments
T_7	Garlic oil
T_8	Ginger oil
T_9	Piper oil
T_{10}	Control (untreated)

Plate 4.17. *In vitro* efficacy of essential oils against *X. campestris* pv. *mangiferaeindicae*

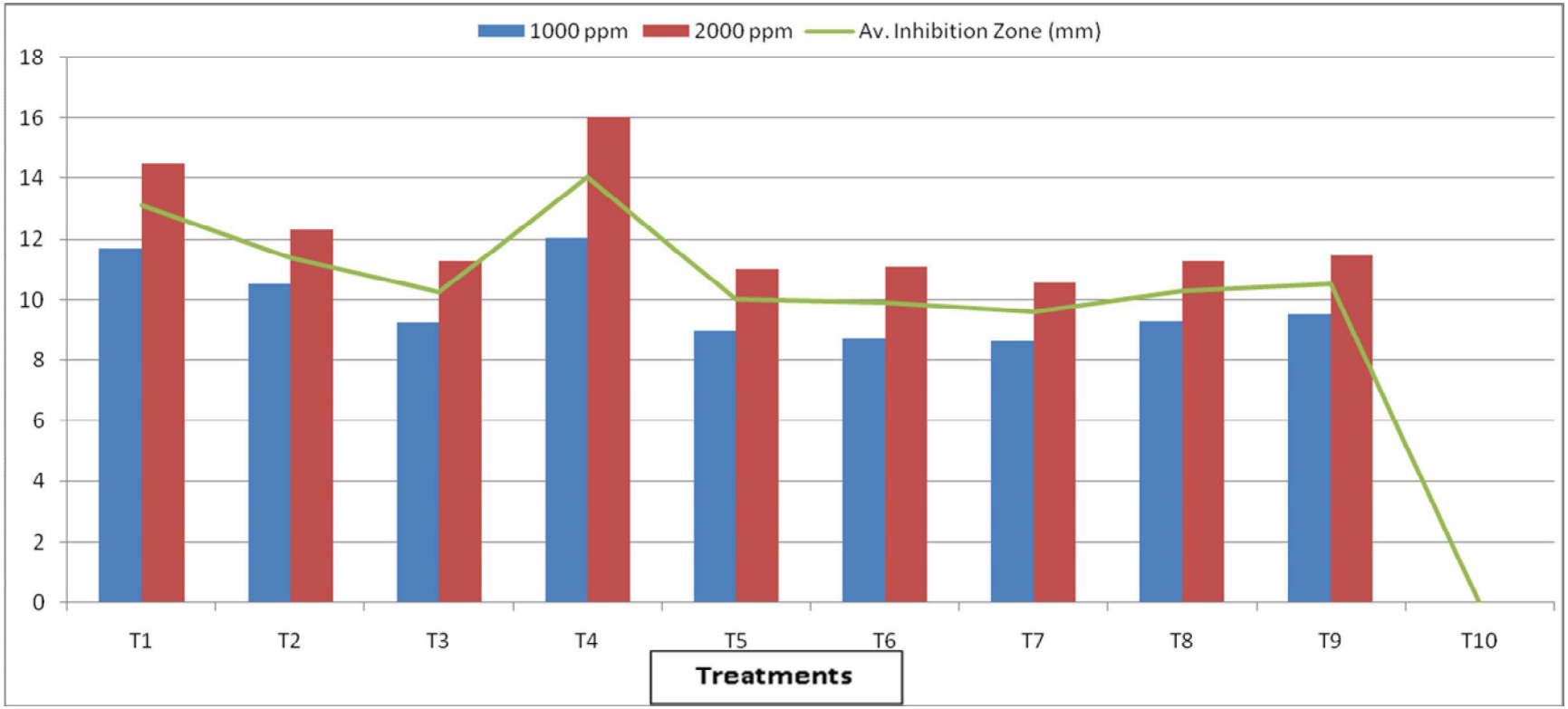


Fig. 4.20. ***In vitro*** **efficacy of essential oils against** ***X. campestris*** **pv.** ***mangiferaeindicae***

T_1	Neem oil	T_6	Mentha oil
T_2	Citronella oil	T_7	Garlic oil
T_3	Clove oil	T_8	Ginger oil
T_4	Cinnamon oil	T_9	Piper oil
T_5	Eucalyptus oil	T_{10}	Control (untreated)

At 1000 ppm, bacterial mean inhibition zone ranged from 8.65 mm (Garlic oil) to 12.04 mm (Cinnamon oil). However, it was significantly highest with Cinnamon oil (12.04 mm), followed by Neem oil (11.70 mm) but both are at par with each other, Citronella oil (10.50 mm), Piper oil (9.52 mm) but were at par with each other, Ginger oil (9.28 mm), Clove oil (9.26 mm), Eucalyptus oil (9.00 mm), Mentha oil (8.70 mm) but were at par with Paper oil (9.52 mm); whereas, significantly least inhibition zone was found with Garlic oil (8.65 mm).

At 2000 ppm, bacterial mean inhibition zone was ranged from 10.55 mm (Garlic oil) to 16.02 mm (Cinnamon oil). However, it was significantly highest with Cinnamon oil (16.02 mm) and found most superior, followed by Neem oil (14.50 mm) and Citronella oil (12.30 mm); whereas, significantly least mean inhibition zone found with Garlic oil (10.55 mm). While, rest most of all treatments were at par i.e Piper oil (11.48 mm), Ginger oil (11.28 mm), Clove oil (11.26 mm), Mentha oil (11.10 mm) and Eucalyptus oil (11.00 mm).

Average inhibition zone was ranged from 9.60 mm (Garlic oil) to 14.03 mm (Cinnamon oil). However, it was significantly highest with Cinnamon oil (14.03 mm) and found most superior, followed by Neem oil (13.10 mm), Citronella oil (11.40 mm), Paper oil (10.50 mm), Ginger oil (10.28 mm), Clove oil (10.25 mm), Eucalyptus oil (10.00 mm) Mentha oil (9.90 mm); whereas least average inhibition zone was found with Garlic oil (9.63 mm).

Similar findings on bioefficacy of essential oils *Xanthomonas* spp. were also recorded earlier by many workers. (Kizil *et al*. 2005; Kotan *et al.* 2007; Paret *et al*. 2010; Mikicinski *et al*. 2012; Gakuubi *et al*. 2016; Benchouikh *et al.,*2016). Lucas *et al.* (2012) studied *in vitro* antimicrobial activity of total seven plant essential oils derived from citronella, clove, cinammone, lemon grass, eucalyptus, thyme and tea tree each @ 0.1, 1.0, 10 and 100 per cent concentrations in 1% powder milk against *Xanthomonas vesicatoria,* using disc diffusion method. Study revealed that, none of the essential oil enhibited growth of *Xanthomonas vesicatoria,* at concentration of 0.1%, while the essential oils of thyme, clove and cinammone partially inhibited the growth of bacterium at the concentration of 1%. However, at the concentrations of 10 and 100% all of the essential oils inhibited bacterial growth. Wonni *et al.* (2016) studied effect of total tree plant essential oils

viz., symbopogon citrates, eucalyptus camaldulensis, mentha piperita against two pathovar of *Xanthomonas oryzae* attacking rice *in vitro* by agar diffusion method. Study revealed that all tested essential oils inhibited considerable antibacterial activity against both pathovar of *X. oryzae.* Essential oils from *C. citrates* found most effective against *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola* with inhibition diameter 34.33mm, followed by *E. camaldulensis* 12.67mm; least inhibition found with *M. piperita i.e* 10.22mm.

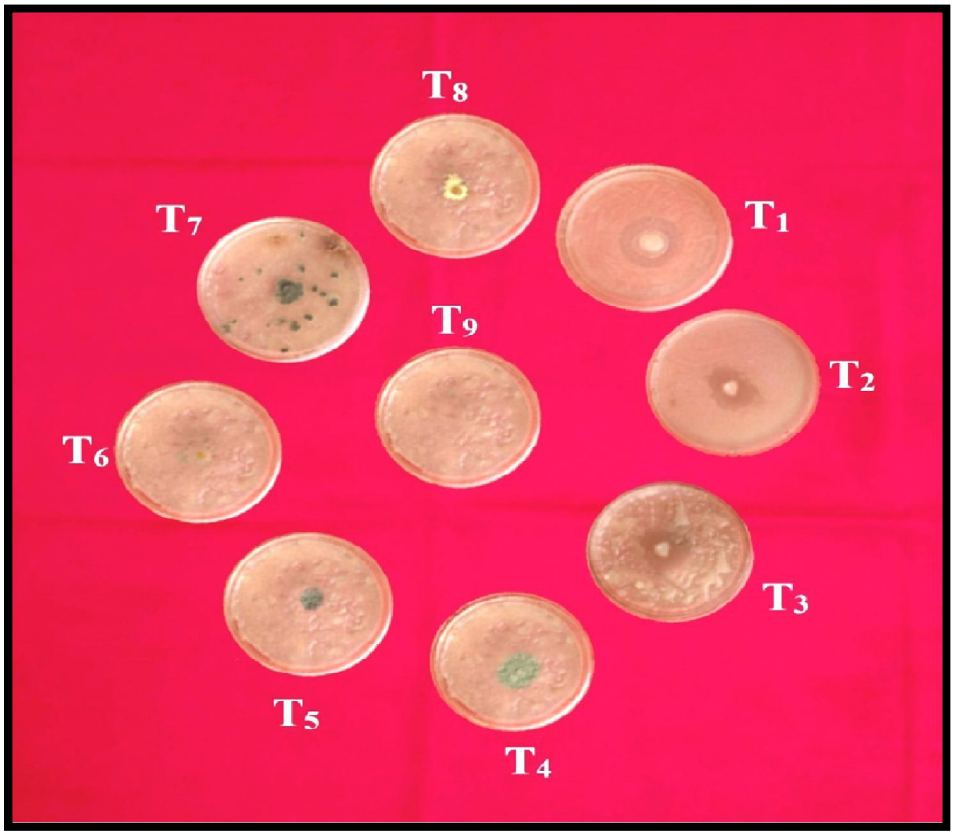
4.9.4. *In vitro* efficacy of bioagents against *X. campestris* pv. *mangiferaeindicae*

Antibacterial activity of total eight antagonists was evaluated in vitro by inhibition zone assay method. The results obtained so inhibition zone of the test bacterium are presented (Table 4.32, Plate 4.18 & Fig. 4.21).

Table 4.32: *In vitro* efficacy of bioagents against *X. campestris* pv. *mangiferaeindicae*

Tr. No.	Treatments	Mean Inhibition Zone (mm)*
T_1	*Bacillus subtilis*	11.80
T_2	*Psuedomonas fluorescens*	15.30
T_3	*P. striata*	10.70
T_4	*Trichoderma harzianum*	9.00
T_5	*T. viride*	8.50
T_6	*Verticillium lecanii*	6.00
T_7	*T. hamatum*	7.20
T_8	*T. virens*	7.35
T_9	Control (untreated)	0.00
	S.E. ±	**0.26**
	C.D. (P=0.01)	**0.71**
*Mean of three replications Fingers in parenthesis are square root transformed values		

Results indicated that, the bioagents tested, significantly inhibited growth of *X. campestris* pv. *mangiferaeindicae*, over control (untreated). However, mean inhibition zone was ranged from 6.00 mm (*V. lecanii*) to 15.30 mm (*P. fluorescens*). However, it was significantly highest with *P. fluorescens* (15.30 mm), followed by *B. subtilis* (11.80 mm), *P. striata* (10.70 mm), *T. harzianum* (9.00 mm), *T. viride* (8.50 mm) but were at par with each other, *T. virens* (7.35 mm) and *T.*



Tr. No.	Treatments
T_1	*Bacillus subtilis*
T_2	*Psuedomonas fluorescens*
T_3	*P. striata*
T_4	*Trichoderma harzianum*
T_5	*T. viride*
T_6	*Verticillium lecanii*
T_7	*T. namatum*
T_8	*T. virens*
T_9	Control (untreated)

Plate 4.18. *In vitro* efficacy of bioagents against *X. campestris* pv. *mangiferaeindicae*

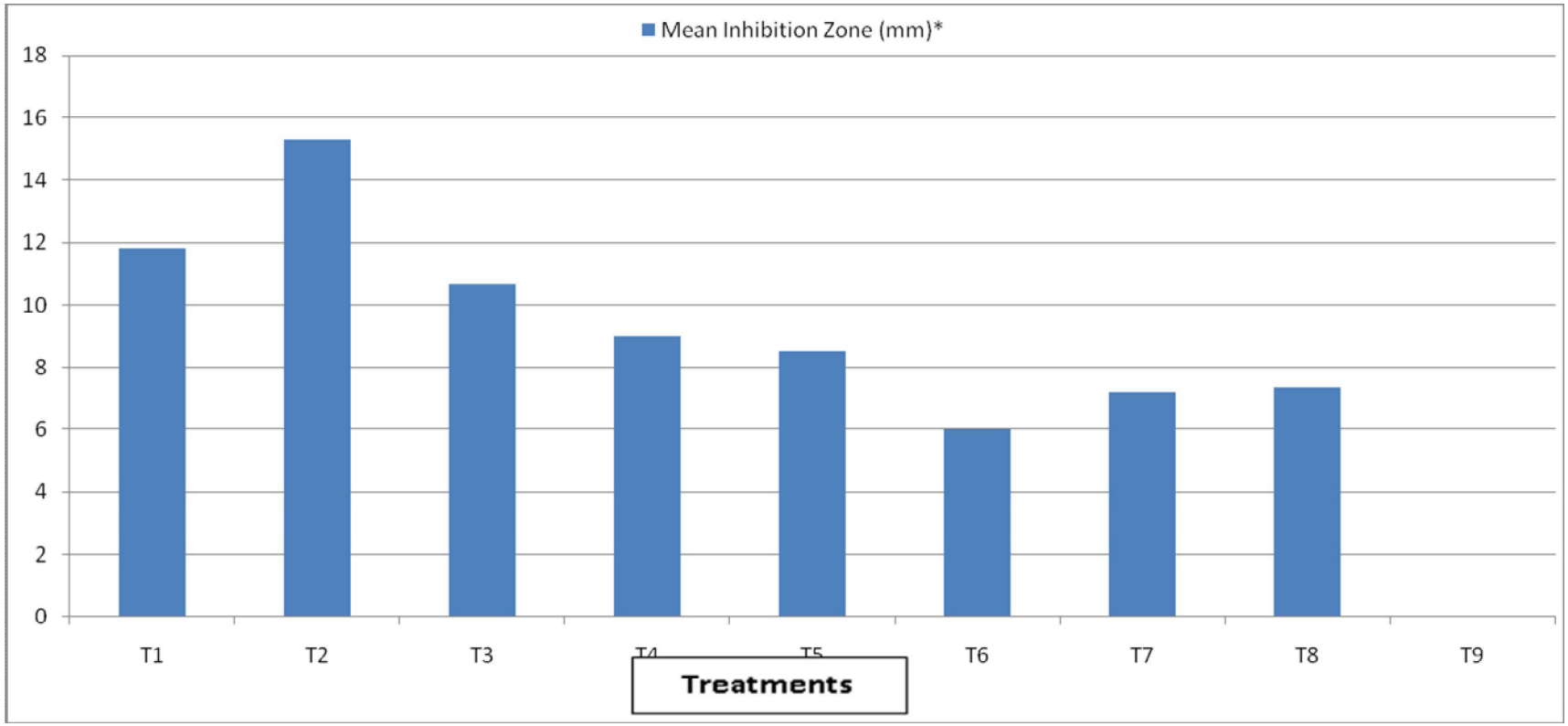


Fig. 4.21. ***In vitro*** **efficacy of bioagents against** ***X. campestris*** **pv.** ***mangiferaeindicae***

T_1	*Bacillus subtilis*	T_6	*Verticillium lecanii*
T_2	*Psuedomonas fluorescens*	T_7	*T. hamatum*
T_3	*P. striata*	T_8	*T. virens*
T_4	*Trichoderma harzianum*	T_9	Control (untreated)
T_5	*T. viride*		

hamatum (7.20 mm) both were at par with each other; whereas, significantly least inhibition zone was found with *V. lecanii* (6.00mm).

Similar finding were also reported by many workers. Jambenal *et al.,* (2011) tested *in vitro* efficacy of two bioagents namely *Pseudomonas flourescens* and *Bacillus subtilis* against *X. campestris* pv. *viticola* causing bacterial leaf spot of grape. Among them, *P. flouresens* recorded highest inhibition of radial growth (8.15 mm) than *Bacillus subtilis* (7.05 mm). Thirumalesh (2012) evaluated two bioagents *Trichoderma* sp. And *Lactobacillus* sp. *In vitro* against *X. campestris* pv. *mangiferaeindicae* causing mango bacterial leaf spot. Among them, *Trichoderma* sp. Produced maximum inhibition zone of 3.00 mm and *lactobacillus* sp. showed inhibition. Katwal (2015) reported the inhibiting effect of *P. flourescens* (12.80 mm) with maximum inhibition zone followed by *B. subtilis* (10.22 mm) and *Trichoderma viride* (6.64 mm) tested *in vitro* against *X. axonopodis* pv. *pinicae* causing bacterial blight of pomegranate. Patil *et al.* (2017) reported *T. harzianum* was found significantly superior in inhibiting the growth of pathogen (22.86 mm) followed by *P. flourescens* (17.20 mm) against *X. oryzae* pv. *oryzae* causing bacterial leaf blight of rice. Naik *et al.* (2018) tested *in vitro* efficacy of different bioagents against *X. campestris* pv. *arecae* causing bacterial leaf stripe of arecanut, among them *T. harzianum* (24.10 mm) was found significantly superior in inhibiting the growth of pathogen followed by *P. flourescens* (15.70 mm).

4.9.5. Disease management in green house (Pot culture)

A total eleven treatments, comprising three antagonists, three antibiotics, three antibacterial fungicides and two essential oils found most effective against the *X. campestris* pv. *mangiferaeindicae* during present *in vitro* studies were selected and assessed for management of bacterial leaf spot of mango (Pot culture). The results obtained on per cent disease intensity are presented in Table 4.33, Plate 4.19 & Fig. 4.22.

All antibiotics, fungicides, bioagents and essential oils tested (pot culture) against *X. campestris* pv. *mangiferaeindicae* significantly reduced the disease intensity after first and second spraying over control. However, Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w found most effective with lowest PDI

mean 34.07 per cent, The second and third best controls recorded were Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (40.30 %) and Kasugamycin 5%+ Copper oxycloride 45% WP (42.15 %); whereas, it was highest with *P. striata* (48.77 %) and found least superior. Mean per cent disease control (PDC) was achieved with all the treatments. However, highest mean per cent disease control of was recorded with Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w (44.01 %), followed by Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (34.57 %); whereas, it was minimum with *P. striata* (21.13 %).

Table 4.33: Effect of various treatments on bacterial leaf spot disease intensity on Mango (Pot culture)

Tr. No	Treatments	PDI before spraying	PDI after spraying		Mean PDI	PDC after spraying		Mean PDC
			First	Second		First	Second	
T_1	*Psuedomonas fluorescens*	45.66 (42.51)	50.80 (45.45)	43.15 (41.06)	46.98	13.45	34.77	24.11
T_2	*Bacillus subtilis*	45.86 (42.62)	52.30 (46.31)	45.50 (42.41)	48.90	10.90	31.22	21.06
T_3	*Psuedomonas striata*	48 (43.85)	53.55 (47.03)	44.00 (41.55)	48.77	8.77	33.49	21.13
T_4	Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w (Streptocycline)	44.33 (41.74)	46.60 (43.05)	21.55 (27.66)	34.07	20.61	67.42	44.01
T_5	Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (Plantomycin)	43.90 (41.49)	47.00 (43.28)	33.60 (35.42)	40.30	11.93	49.21	34.57
T_6	Kasugamycin 5%+ Copper oxycloride 45% WP(Conika)	44.30 (41.72)	46.56 (43.02)	37.75 (37.91)	42.15	20.68	42.93	31.80
T_7	Copper oxychloride 50% WP	45.00 (42.13)	48.60 (44.19)	38.60 (38.41)	43.60	17.20	41.65	29.42
T_8	Mancozeb 75% WP	45.40 (42.36)	50.58 (45.33)	40.10 (39.29)	45.34	13.83	39.38	26.60
T_9	Cymoxanil 8% + Mancozeb 64% WP	44.60 (41.89)	51.90 (46.08)	34.40 (35.97)	43.15	11.58	48.00	29.80
T_{10}	Cinamon oil	44.90 (42.51)	49.58 (44.76)	38.80 (38.52)	44.19	15.53	41.35	28.44
T_{11}	Neem oil	43.80	50.60	39.40	45.00	13.80	40.44	27.12

Plate 4.19. Efficacy of various treatments on bacterial leaf spot disease intensity on mango (Pot culture)

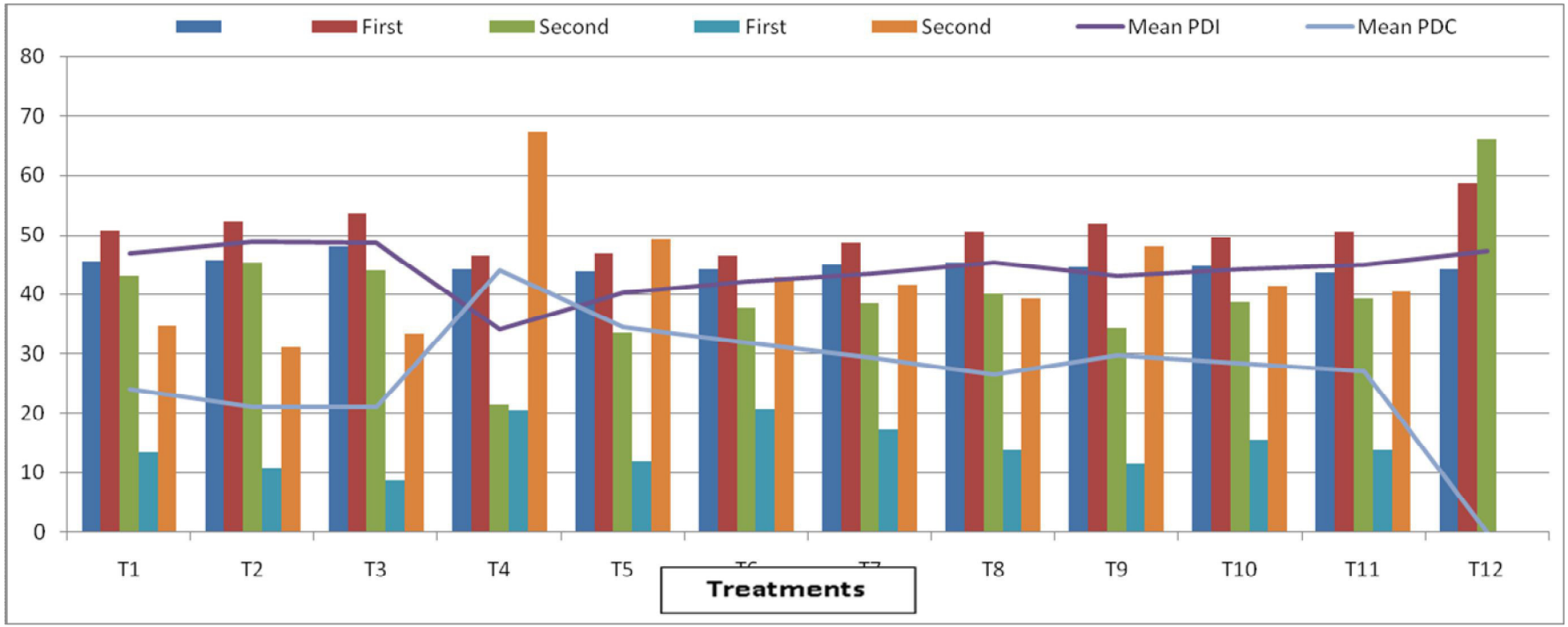


Fig. 4.22. Effects of various treatments on bacterial leaf spot disease intensity on mango (pot culture)

T_1	*Psuedomonas fluorescens*	T_7	Copper oxychloride 50% WP
T_2	*Bacillus subtilis*	T_8	Mancozeb 75% WP
T_3	*Psuedomonas striata*	T_9	Cymoxanil 8% + Mancozeb 64% WP
T_4	Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w (Streptocycline)	T_{10}	Cinamon oil
T_5	Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (Plantomycin)	T_{11}	Neem oil
T_6	Kasugamycin 5%+ Copper oxycloride 45% WP(Conika)	T_{12}	Control

		(41.43)	(45.34)	(38.88)				
T_{12}	Control	44.33 (12.01)	58.70 (50.01)	66.15 (54.42)	47.42	0.00	0.00	0.00
	S.E. ±	**0.27**	**0.33**	**0.35**	--	--	--	--
	C.D. (P=0.01)	**0.82**	**0.98**	**1.08**	--	--	--	--

*Mean of three replications
Figures in parentheses are arcsine transformed values
PDI: - Percent disease intensity

These results obtained in present study are in accordance with the reports of many earlier workers. Giri *et al.* (2008) evaluated *in vitro* three bioagents *viz., T. harzianum, P. fluorescens* and *A. niger* against *X. axonopodis* pv. *citri* causing citrus canker. Results revealed that, most effective antagonist was *A. niger* with 17.69 per cent disease control followed by *P. fluorescens* (16.67%). The least effective antagonist among them was *T. harzianum* exhibitated 15.30 per cent disease control. Thirumalesh *et al.,* (2012) studied five compounds reduce symptoms caused by *X. campestris* pv. *mangiferaeindicae* was evaluated in plants sprayed with the pathogen. Symptoms, like black spots, appeared in inoculated plants 1 week after inoculation, among inoculated controls and plants treated with bavistin, some displayed leaf spots. copper oxychloride + copper sulphate; streptocycline + bactrinashak; mancozeb + copper oxychloride, mancozeb + bavistin and bavistin + bactrinashak significantly reduced ($p<0.05$) the percentage of affected leaves, The more reduced leaf symptoms by applying streptocycline + copper sulphate and by streptocycline. These compounds reduced black spots, compared with inoculated controls. For copper oxychloride + copper sulphate; streptocycline + bactrinashak; mancozeb + copper oxychloride, mancozeb + bavistin and bavistin + bactrinashak this reduction was significant. *Xcm* was not easily isolated more than 70% of inoculated plants in samples obtained from symptomatic leaves and black spots, which was treated with copper oxychloride + copper sulphate; streptocycline + bactrinashak; mancozeb + copper oxychloride, mancozeb + bavistin and bavistin + bactrinashak treatment in two independent experiments. Jadhav (2019) tested antibiotics, fungicides, bioagents and essential oils (pot culture) against *X. axonopodis* pv. *citri* significantly reduced the disease intensity after first and second spraying over control. However, streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w found most effective with lowest PDI meab 34.19 per cent. The second and third best controls recorded were streptomycin sulphate 90% + tetracycline hydrochloride 1 % w/w (40.83) and kasugamycin 5% + copper oxychloride 45% WP (42.33); whereas, it was highest with *P. striata* (PDI

49.34) and found least superior. Mean per cent disease control (PDC) was recorded with streptomycin suphate 90% + tetracycline hydrochloride 10% w/w (43.89), followed by streptomycin sulphate 90%+ tetracycline hydrochloride 1 % w/w (33.34); whereas, it was minimum with *P. striata* (19.81).

CHAPTER-V

SUMMARY AND CONCLUSION

CHAPTER - V

SUMMARY AND CONCLUSIONS

The Mango is a National fruit of India. It is an important fruit crop and grown in varying tropical or subtropical regions in the world. Besides delicious taste, it has excellent flavour and attractive fragrance, it contains a variety of nutrients and rich in vitamin A & C. India ranks first in the production, consumption and export of mango all over the world with an area, production and productivity of 2262.8 ha, 19686.9 t and 8.7 mt/ha respectively whereas, Maharashtra occupies an area of 157.07 ha, production 520.87 t and productivity of 3.58 mt/ha. Mango bacterial leaf spot disease which is also known as mango canker, bacterial spot, bacterial canker, black spot, mango blight, bacterial black spot is caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (*Xcmi*). It is one of the most destructive bacterial disease of mango worldwide. The disease is most serious in areas of high temperature (14-38^{0}C) and high rainfall (more than 1000 mm per year); during the growing season.

Many commercial cultivars are highly susceptible to bacterial leaf spot and infections can result in drastic yield losses associated with premature fruit drop, reduction of fruit quality, and induction of severe defoliation especially when storms or hurricanes are involved. From 50 to 80% fruit infection is common on very susceptible cultivars.

Therefore, present investigations on bacterial leaf spot of mango caused by *X. campestris* pv. *mangiferaeindicae*, were undertaken with the objectives defined and the findings thereof are being summarized in the following paragraphs.

Collection of mango bacterial leaf spot diseased samples were carried out during 2018-19 from three agro-climatic zones of Marathwada of Maharashtra. Results revealed that, bacterial leaf spot of mango is of common occurrence in all agro-climatic zones of Marathwada region exhibiting different per cent intensity of bacterial leaf spot on mango plants irrespective of varieties under cultivation. However, maximum per cent intensity was recorded in Anandwadi (45.30%) from Scarcity zone whereas; minimum per cent intensity was recorded in Kandhar (20.11%) from assured rainfall zone.

Symptoms were observed on leaves, fruits, branches and twigs. Initially small, water soaked lesions delineated by veins were noticed on lower surface of the leaves followed by appearance of small brown to dark brown coloured spots on the upper surface with circular to irregular shape with chlorotic halo. On developing green fruits, symptoms were noticed as small pin head sized, black lesions with diffused water soaked margin, which later developed into black coloured, medium to big sized erumpent cankerous spots with sometimes showing characteristics tear shining symptoms with bacterial exude.

The test bacterium (*X. campestris* pv. *mangiferaeindicae*) was successfully isolated by bacterial ooze obtained from the leaves of naturally infected mango plants, on the selective culture media NA. Total eight virulent isolates of *X. campetris* pv. *mangiferaeindicae* on the basis of pathogenecity test were selected from 35 isolates and assigned them the nomenclature as: Xcm1, Xcm2, Xcm3, Xcm4, Xcm5, Xcm6, Xcm7, Xcm8. The culture of these isolates were purified by single colony isolation technique and transferred on NA slant and stored at 28 ± 2 °C temp. and maintained separately for further *in vitro* studies.

Pathogenicity of *X. campestris* pv. *mangiferaeindicae* causing bacterial leaf spot of mango were proved by applying attached leaf assay in polybag/ pot culture, under screen house conditions, by planting seedlings of mango cultivar Kesar susceptible to bacterial leaf spot disease. Symptoms appeared by artificially inoculated leaves were same as natural appeared, re-isolation of the pathogen was done and culture so obtained was compared with the original culture for the confirmation of Koch's postulate. Thus all isolates confirmed bacterial leaf spot disease on the inoculated plant.

Collected diseased samples expressed a wide range of variability in respect of lesion shape, size and colour etc. The lesion shape varied from circular to irregular. Size of leaf lesions was also varied, ranged from 1 mm to 3 mm in diameter, maximum lesion size was found in Nanded (Xcm7) isolate (3mm). However, minimum lesion size was found in isolates Xcm5 and Xcm6 collected from Assured rainfall zone *i.e* 1 mm. Colour of lesion was mostly dark brown with yellow halo.

Pathogenic variability among the eight isolates of *X. campestris* pv. *mangiferaeindiace* collected from different agro climatic zones of Marathwada region

was detected by two separate methods *viz.,* attached leaf assay and detached leaf assay.

Again these eight different isolates collected from Marathwada region were subjected to pathogenic variability in attached leaf assay under screen house condition. Entire eight test isolates of *X. campestris* pv. *mangiferaeindicae* found pathogenic and caused bacterial leaf spot in mango (Kesar). Average incubation period (days) varied from 12.33 (AR) to 14 (MR). However, maximum incubation period was recorded in isolate Xcm7 from agro-climatic zone MR (14) followed by Xcm4 (13.33) AR. While minimum average incubation period (days) was recorded in AR zone *i.e* 12. Xcm7 isolate had more lesion size i.e. 2.5mm after 14 average incubation days of inoculation followed by Xcm5 which showed 2.26 mm lesion size after 12.33 average incubation days of inoculation and 3.66 average number of spots. The Xcm2 isolate showed less lesion size i.e. 1.66 mm after 12 average incubation days of inoculation showed 2.66 average numbers of spots.

In detached leaf technique under controlled lab conditions, leaves of total six varieties of mango *viz.,* Local, Kesar, Dasheri, Neelam, Amrapali and Alphonso were used and observation were recorded on incubation period and symptom type. The entire eight test isolates of *X. campestris* pv. *mangiferaeindicae* found pathogenic and caused bacterial leaf spot in leaves of all mango cultivars. Minimum incubation period was recorded in cultivar Kesar with isolate Xcm1 (9 days) while, maximum incubation period was recorded in cultivar Alphonso with isolate Xcm6 *i.e.* 16.33 days. Same pattern of symptom expression was found in all the isolates among six cultivars. Symptom expression exhibited by different isolates were grouped under four categories viz., (-) no leaf spot, (+) less leaf spot, (++) moderate leaf spot and (+++) strong leaf spot. Different pattern of symptom expression was found in all the isolates among six cultivars. However, Local and Kesar cultivar showed moderate and severe bacterial leaf spot, while rest all cultivars *viz.,* Dasheri, Neelam, Amrapali and Alphanso showed weak leaf spot.

Cultural characteristics *viz.,* pigmentation, colony shape, elevation, margin, surface appearance and cell shape of different eight test isolates were studied using NA as basal culture medium. Results revealed that, of the eight isolates tested, six isolates *viz.,* Xcm1, Xcm3, Xcm4, Xcm6, Xcm7 and Xcm8 exhibited cream to

white pigmentation while rest two (Xcm2 and Xcm5), exhibited white to pale yellow pigmentation. All the isolates had filiform colony shape, convex elevation and entire colony margin. However, all the isolates were morphologically single rods.

Biochemical characters of *X. campestris* pv. *mangiferaeindicae* were studied by subjecting the bacterial isolated to various tests. Study revealed that all the eight isolates were found negative to Gram's reaction, anaerobic growth test, gelatine liquefication test and tetrazolium salt tolerance test while, positive to KOH test, catalase tes, starch hydrolysis test, casein hydrolysis and xanthum gum production test.

Molecular variability studied among eight isolates of *X. campestris* pv. *mangiferaeindicae* by RAPD – PCR using OPB primers revealed that average polymorphism in strains of *X. campestris* pv. *mangiferaeindicae* based on RAPD analysis was 96.00 %. However, 100 % of polymorphism was reported in primer OPB-6, OPB-17, OPB-19 and OPB-20; whereas, minimum polymorphism was observed in OPB-16 i.e 80.00 %. Dendrogram generated based on UPGMA analysis of RAPD data grouped all these isolates in to two major clusters (Figure 9). These clusters were formed on the basis of genus as well as species level. The group I, comprised, maximum of four isolates together which showed 56.40 % genetic similarity *viz*., Xcm1, Xcm2, Xcm3 and Xcm4. The cluster I comprised isolates of Xcm-2 and Xcm-4 showed that higher similarity each other i.e. 80 %. Cluster II comprised four isolates together showing 57.2 % similarity with each other viz., Xcm-5, Xcm-06, Xcm-07 and Xcm-08. These cluster comprised isolates of Xcm-07 and Xam-08 showed higher similarity each other i.e. 75 %. The cluster I and II comprised of eight isolates showing that 51 % similarity among each other.

In the host range study, leaves of different nine fruit crops were subjected to inoculation of test pathogen. The result revealed that the symptoms of the disease were not observed in all the tested fruit crops. The bacterium is a potential threat to infect the mango crop.

Effect of different age of leaf on disease development (On detached leaves) showed that older leaves were more susceptible than mid age and younger leaves. Older leaves required minimum days for initiation of symptoms (9 days) followed by mid age leaf (10 days), whereas younger leaves didn't show any

symptoms. Older leaves showed about 5.33 average number of spots, while mid age leaves showed average 2 number of spots. Symptoms observed on mid age leaves were Water soaked circular to irregular, brown spots and on old age leaves were Water soaked circular to irregular, dark brown spots with yellow halo.

Correlation between disease and weather factors on fruit revealed that, maximum temperature had significant positive correlation with the disease development in both the years. Role of minimum temperature was significant and positive with the disease development. Relative humidity had negative significant relationship with the disease in both the years while, rainfall had positive significant relationship in the year 2018 and negative significant relationship in the year 2019 with the disease. Correlation between disease and weather factors on leaves revealed that, maximum temperature had significant positive correlation with the disease development in both the years. Role of minimum temperature was significant and positive with the disease development. Relative humidity had positive significant relationship with the disease in both the years. rainfall had positive significant relationship with the disease development in both the years.

Stepwise regression models showed strong and positive relationship between combined effects of weather factors for disease development. The apparent infection rate of the disease was slow initially, which gradually increased when temperature and humidity became favorable in both the years. The area under disease progress curve (AUDPC) was more on leaves as compared to fruits in both the years.

The antibiotics tested at various concentrations (each @ RD, 50% RD and 125% RD) significantly inhibited growth of *X. campestris* pv. *mangiferaeindicae,* over control. Average inhibition zone was ranged from 10.80 mm (Biomycin) to 15.30 mm (Streptocycline). However, it was significantly higher with 15.30 mm (Streptocycline), followed by Plantomycin (13.50 mm) and Bacterinol + Copper oxychlorode (12.66 mm), Conika (12.15 mm) and Bacterinol (11.88 mm): whereas, significantly least inhibition zone was found with Biomycin (10.80 mm).

The fungicides tested at various concentrations (each @ RD, 50% RD and 125% RD) significantly inhibited growth of *X. campestris* pv. *mangiferaeindicae,* over control. Average inhibition zone ranged from 8.75 mm (Carbendazim 50% WP) to 12.98 mm (Copper oxychloride 50% WP). However, it was significantly highest

with Copper oxychloride 50% WP (12.98 mm) followed by Cyamoxanil 8% + Mancozeb 75 % WP (11.95), Mancozeb 75 % WP (11.50), Chlorothalonil 75 % WP (10.90 mm); whereas, significantly least inhibition zone was found with Carbendazim 50 % WP(8.75 mm).

Antibacterial activity of total nine essential oils tested at various concentrations (each @ RD, 50% RD and 125% RD) significantly inhibited growth of *X. campestris* pv. *mangiferaeindicae,* over control. Average inhibition zone was ranged from 9.60 mm (Garlic oil) to 14.03 mm (Cinnamon oil). However, it was significantly highest with Cinnamon oil (14.03 mm) and found most superior, followed by Neem oil (13.10 mm), Citronella oil (11.40 mm), Paper oil (10.50 mm); whereas least average inhibition zone was found with Garlic oil (9.63 mm).

The bioagents tested against *X. campestris* pv. *mangiferaeindicae* significantly inhibited the growth over control. However, mean inhibition zone was ranged from 6.00 mm (*V. lecanii*) to 15.30 mm (*P. fluorescens*). However, it was significantly highest with *P. fluorescens* (15.30 mm), followed by *B. subtilis* (11.80 mm), whereas, significantly least inhibition zone was found with *V. lecanii* (6.00mm).

All antibiotics, fungicides, bioagents and essential oils tested (pot culture) against *X. campestris* pv. *mangiferaeindicae* significantly reduced the disease intensity after first and second spraying over control. However, Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w found most effective with lowest PDI (34.07 %), The second and third best controls recorded were Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (40.30 %) and Kasugamycin 5%+ Copper oxycloride 45% WP (42.15 %); whereas, it was highest with *P. striata* (48.77 %) and found least superior. Mean per cent disease control (PDC) was achieved with all the treatments. However, highest mean per cent disease control of was recorded with Streptomycin sulphate 90% + Tetracycline hydrochloride 10% w/w (44.01 %), followed by Streptomycin sulphate 9% + Tetracycline hydrochloride 1% w/w (34.57 %); whereas, it was minimum with *Pseudomonas striata* (21.13 %).

CONCLUSIONS

- ✓ Bacterial leaf spot of mango is of common occurrence and widely distributed in all agro-climatic zones of Marathwada region and it was more severe in Assured rainfall zone. Also the disease was observed throughout the year on different plant parts.

- ✓ The pathogen *X. campestris* pv. *mangiferaeindicae* was successfully isolated on nutrient agar from naturally infected diseased leaves, fruits and twigs specimens of mango collected and about eight isolates, one each representative of eight district of Marathwada were obtained, purified and used. Pathogenicity of all test isolates was successfully proved on mango cultivars under screen house as well as under lab conditions.

- ✓ All eight isolates of *X. campestris* pv. *mangiferaeindicae* representing eight districts of Marathwada region of three agro-climatic zones of Maharashtra state exhibited a wide range of pathogenic variability on mango plants as well as on detached leaves in controlled lab conditions.

- ✓ Based on symptomatology, cultural and morphological characters, microscopic observations, gram staining and pathogenecity the test pathogen was identified and confirmed as *Xanthomonas campestris* pv. *mangiferaeindicae*.

- ✓ All the eight isolates of *X. campestris* pv. *mangiferaeindicae* exhibited a wide range of symptomatic and molecular variability which indicated that the possibility of existence of variables amongst the population in the Marathwada region.

- ✓ Out of six cultivars of mango, cv. Local and Keshar were highly susceptible and remaining four *viz.,* Dasheri, Neelam, Amrapali and Alphonso were moderately susceptible to bacterial leaf spot.

- ✓ The apparent infection rate of the disease was slow initially, which increases when weather conditions were favourable.

- ✓ The area under disease progress curve (AUDPC) was more on leaves as compared to fruits in both the years.

- ✓ Rainfall followed by average temperature and high humidity favours the disease.

- ✓ All the antibiotics significantly inhibited the growth of test pathogen *in vitro*. However, Streptocycline was found most superior with significantly highest inhibition of test pathogen. The second best antibiotic found was Plantomycin with comparatively low inhibition of test pathogen.

- ✓ Amongst all fungicides tested *in vitro*, Copper oxychloride 50% WP found most superior with highest inhibition of test pathogen, followed by Cyamoxanil 8% + Mancozeb 75% WP with comparatively low inhibition of test pathogen.

- ✓ All essential oils were found antibacterial. However, Cinnamon oil was found most superior with highest inhibition of test pathogen. Second best oil found was Neem oil with comparatively low inhibition of test pathogen.

- ✓ All the antagonists tested *in vitro* inhibited growth of pathogen. However, *Psuedomonas fluorescens* inhibited highest growth of test pathogen and found most superior amongst all.

- ✓ All the antibiotics, antibacterial fungicides, essential oils and antagonists tested against bacterial leaf spot disease in pot culture, significantly reduced per cent disease intensity. However, Streptocycline was found most superior with maximum mean per cent disease control.

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APPENDICES

APPENDIX -I

Composition of various culture media used for *in vitro* studies

1) Nutrient agar

Beef extract : 03 g

Peptone : 05 g

Agar agar : 20 g

Distilled water : 1000 ml

2) Nutrient broth

Beef extract : 03 g

Peptone : 05 g

Distilled water : 1000 ml

APPENDIX -II

Composition of various buffer, solutions and reagents used for molecular work

1 Stocks solution for DNA isolation

Tris HCl (1M, pH 8.5)	:	Tris base 15.76 gm in 100 ml distilled water.
NaCl (5M)	:	NaCl 29.22 gm in 100 ml distilled water.
EDTA (0.5M, pH 8.0)	:	EDTA 18.61 gm in 100 ml distilled water.
P:C:I (25:24:1)	:	Add Phenol 25 ml, Chloroform 24 ml and Isoamyl alcohol 1 ml.
Sodium acetate (3M)	:	Sodium acetate 24.60 g in 100 ml distilled water.
Ammonium acetate (7.5M)	:	Ammonium acetate 57.75 gm in 100 ml distilled water

2 DNA extraction buffer (50 ml)

Tris HCl (1M)	:	5 ml
NaCl (5M)	:	5 ml
EDTA (0.5M)	:	2 ml
SDS (10%)	:	10 ml
Distilled water	:	28 ml

3. Reagents for agarose gel electrophoresis

a. 50X TAE buffer composition (1L)

Tris base	:	242 g
Acetic acid	:	57.10 ml
Na_2EDTA	:	37.20 g

Dissolve above components into distilled water and make up vol. 1 lit

b. TE buffer composition (100 ml)

Tris HCL	:	10 ml
Na_2EDTA	:	200 µl

c. 6X DNA gel loading dye

Bromophenol blue (BPB)	:	0.25 %
Xylene cyanol (optional)	:	0.25 %
Glycerol in water	:	30 %

d. Ethidium bromide (5 µl/ml)

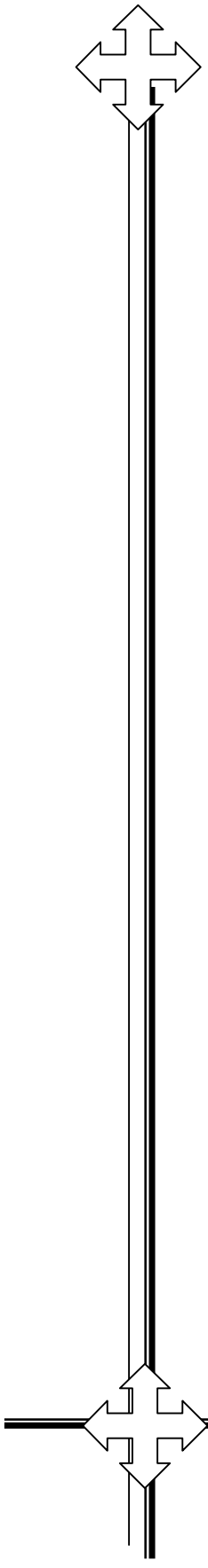
Dissolve 100 mg Ethidium bromide in 10 ml of distilled water.

e. Recipe for 1.2% agarose gel (30 ml)

Agarose	:	360 mg
1x TAE	:	30 ml
(5 µl /ml)	:	5 µl

5. RNase stock (10mg/ml)

Dissolve 10 mg RNase in 1ml of sterile distilled water and store at 20^0C.



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