Chapter- 5

Discussion
DISCUSSION

In the present study pathogen isolated and identified by direct plating method and by liquid assay methods. The casual organism *Xanthomonas campestris* pv. *mangiferaeindicae* was identified by morphological, biochemical, physiological and pathological tests. The plant parts that showed typical bacterial black spot symptoms were collected from bacterial black spot infested orchards of Mango in Mango growing districts of Karnataka during the survey. Isolates were yielded white to yellow, mucoid, circular, raised 1 to 2 mm colonies on NA and YNA after 48 to 72 hrs of incubation at 30°C. These isolates were then streaked onto YNA slants and all the bacterial isolates showed luxurious growth on YNA and NA. The organism *Xanthomonas campestris* pv. *mangiferaeindicae* were confirmed by morphological, biochemical, physiological and pathological tests.

Cazorla, *et al.*, (1998) have isolated the causal agent of apical necrosis, small pieces of affected leaves, buds, and other tissues of the mango were cut from the edge of a recent necrotic lesion, placed in sterile plastic bags, transported to the laboratory, and processed on the day of sampling. The bacterial isolates were identified according to biochemical, physiological tests and pathogenicity tests. Dayakar and Gnanamanickam, (1996) has purified and maintained the bacterial isolates, bacterial colonies on YNA and NA in sterilized petriplates.

Kishun and Chand, (1988) has proved that the survival of *Xanthomonas campestris* pv. *mangiferaeindicae*, the incitant of bacterial canker of mango on different weeds present in mango orchards. The pathogen survives epiphytically on weed hosts which may play an important role in its disease cycle. The presence of the pathogen in the alternative host varied with season.
In the present study of RAPD analysis Band statistics and cultivar similarity of RAPD markers further confirmed polymorphism among the studied 15 Xcm accessions. Ten RAPD primers generated 106 loci and among which 101 loci (95.28%) were polymorphic and 5 (4.71%) were monomorphic. An average of about 10.6 bands was generated per primer, which is the fragment size varied from 0.2 - 1.5 kb. The number of bands per primer ranged from 8 to 15 with a mean of 10.6 bands per primer. The extent of polymorphism per primer varied from 80.0% to 100% with a mean of 95.28%. The similarity values between pairs of accessions ranged between 1.41 and 7.21 with a mean of 4.31. Since RAPD markers randomly scatter throughout the genome, the number of markers matters greatly in analyzing the accessions that would cover the entire genome to a greater extent.

Population analysis dendrogram generated based on the UPGMA analysis from the 15 bacterial genotypes data clearly grouped into two broad clusters at 80.6% dissimilarity. The group 1 contains 8 strains (KUM, DAS, PAV, GOB, PER, BET, MUT, RAYL) and remaining group contains 7 strains (MLK, TVG, BELG, YAR, KRC, BIS, LIN) formed a cluster. The PER and BET strains shared very low variability and formed cluster at 30.6% dissimilarity.

Valverde, et al., (2007) and Klaassen et al., (2002) have studied AFLP, rep-PCR and PFGE was used to examine genetic diversity among X. campestris pv. campestris isolates in 14 isolates obtained in Israel, six isolates representing the six known races of this pathogen and two recently sequenced isolates, Cluster analysis of PFGE and AFLP fingerprints showed a high level of correlation, leading to the detection of 11 and 12 types, respectively, Vorholter, et al., (2003) and Qian, et al., (2005), compared two sequenced genomes compared genetic variation within of X.
campestris pv. campestris revealed significant differences in their gene composition. In Tanzania, Schaad, et al., (2006) showed high level of genetic relatedness with several pathovars of *X. axonopodis* group. Lazo, et al., (1987) cloned deoxyribonucleic acid (DNA) fragments, used to detect restriction fragment-length polymorphism among 93 strains of *X. campestris*, which make up 26 pathovars. Differences among the pathovars were also confirmed by pathogenicity experiments on plants.

Mbwana, et al., (2006) have adapted and optimized the technique of random amplified polymorphic DNA (RAPD) to study *Haemophilus ducreyi* isolates. A panel of 43 strains isolated from chancroid patients from different countries in Africa, Europe, North America, and Asia were characterized. The isolates from Thailand were exceptional in that they showed greater diversity and were represented by six different RAPD patterns. Maiti, et al., (2009) showed that Sixty five isolates of *Vibrio harveyi* were subjected to random amplified polymorphic DNA (RAPD)-PCR analysis of genetic variability among *V. harveyi*. A total of 10 RAPD primers were assayed, the genetic diversity among *V. harveyi* isolates assessed by RAPD-PCR using PM3 primer yielded 35 different RAPD patterns which clustered the isolates into 15 groups at 72% similarity level. Similarly, RAPD-PCR with CRA25 clustered the 38 patterns into 10 groups at 74% similarity. The discriminatory index (D) value calculated for RAPD fingerprints generated with PM3 and CRA25 were 0.90 and 0.85, respectively, Zoysa, and Efstratiou, (1999) find out that the usefulness of random amplification of polymorphic DNA (RAPD) analysis of 45 *Corynebacterium diphtheriae* isolates from Eastern Europe and
neighbouring countries. Twenty RAPD profiles were revealed among the 45 isolates. There was 100% correlation between RAPD profiles and ribotypes.

Hejazi, et al., (1997) shown Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was a convenient typing method for *S. marcescens*. By applying this combination to 175 isolates of *S. marcescens*, which could be classified into 38 groups on the basis of serotyping and phage typing, 73 different RAPD patterns with good reproducibility were obtained.

In the present study culture filtrates of *Trichoderma* sp. inhibit the growth of the *Xanthomonas campestris* pv. *mangiferaeindicae* in *in vitro* conditions performed by well method, whereas *Lactobacillus* sp. isolated from yoghurt was fail to inhibit the organism by the dual culture analysis method.

Yobo, et al., (2009) have conducted shadehouse pot trial efficiency of single and dual inoculations with selected *Trichoderma* and *Bacillus* isolates on performance of dry bean plants. The greatest degree of nodulation was observed in *Trichoderma* and/or *Bacillus* treated plants. Karthikeyan, et al., (2008) have shows three antagonists *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride*, were tested alone and in combination for suppression of onion leaf blight (*Alternaria palandui*) disease under glasshouse and field conditions. The average mean of disease reduction was 24.81% for single strains and 42.44% for mixtures. In addition to disease suppression, treatment with a mixture of antagonists promoted plant growth in terms of increased plant height and ultimately bulb yield, Datnoff, et al., (1995) find the concept of mixture of biocontrol agents for disease control has already been well demonstrated in several crops including apple (Janisiewicz, 1996),
wheat (Pierson, and Weller, 1994), cucumber (Raupach, and Kloeppe, 1998) and
tomato.

Paddera, and Sharma, (2011) has showed Bean anthracnose caused by
Colletotrichum lindemuthianum controlled by bioagents, viz. Trichoderma viride,
Trichoderma harzianum, Trichoderma hamatum and Gliocladium virens conducted
under in vitro and in vivo Culture filtrate from T. viride was found and reduced the
spore germination of test fungus significantly, Mougya, et al., (2011) have studied
simple laboratory techniques to examine the influence of the antagonistic isolates of
Trichoderma harzianum, T. viride, Bacillius subtilis and Pseudomons flourescence
and their culture filtrates on selected soilborne root rot pathogens Rhizoctonia solani
and Fusarium solani. Calistru, et al., (1997) find that culture filtrates of
Trichoderma viride and Trichoderma harzianum was inhibited Fusarium
moniliforme, and Trichoderma spp. is potential candidates for biocontrol of some
mycotoxin-producing fungi.

Walker et al., (1998) showed that technique of dual culture analysis on agar
plates was an easy assay with which to select antagonistic bacteria from a random
group of bacterial isolates and to compare these selected strains for their fungal
displaying a wide antifungal spectrum were selected as the most interesting bacteria
to test for biological control of cucurbit powdery mildew. These four isolates were
characterized as Bacillus spp. The characteristics of Bacillus species as antibiotic
producer and inhabitant of phyllosphere are well known (Foldes, et al., 2000);
therefore, it was not a surprise to isolate different Bacillus species by means of a
screening method based on antibiotic production (Shoda, 2000).
In this investigation the antibacterial compounds are playing a major role in controlling bacterial plant diseases. The commercial bactericides have not been assessed against the causal agent of the disease. The present results of this study, obtained in vitro, showed that the antibiotics ciproflaxacin, tetracycline and kanamycin was the strongest effect against the four tested strains of *Xanthomonas campestris pv. mangiferaeindicae* whereas vancomycin was not effective. Other compounds like gentamycin, chloromphenicol, copper sulphate, copper oxychloride and commercial bactrinashak also exerted in vitro antibacterial activity.

Pruvost, *et al.,* (2005) showed susceptibility to 69 antibiotics was assayed by the disk susceptibility test on Mueller–Hinton agar. Inhibition diameters were recorded after incubation for 48 h at 28°C, By contrast, Okigbo, and Osuinde, (2003), find out streptomycin did reduce leaf symptoms produced by spray inoculation in two independent trials, and the black spots on the leaves and stems in one. *Bacillus subtilis* were considerably reduced in the field by the application of the antagonist, differences in disease control according to the inoculation method (Hausbeck, *et al.,* 2000) reported that streptomycin applied to seedlings inoculated by misting increased their survival after transplant and prevented severe disease symptoms from developing in the field, Mishra and Prakash, (1992) showed streptocycline was best chemical for control.

Our study revealed that copper sulphate combined with other commercial formulates and copper sulphate alone was the most effective treatment in reducing symptoms in plants inoculated with *Xcm* and synergistic effects of copper sulphate was effective alone and along with other commercial formultes against *Xcm*. in *vitro* and in green house conditions by spraying. A selection of antimicrobial
compounds was made based on the *in vitro* results for *in vivo* assays in greenhouse-grown mango plants inoculated by spraying and pricking. For both inoculation methods, typical reproducible symptoms were visible 1 week after inoculation. *In vivo* assays revealed that pricking inoculation with *Xcm* produced symptoms of systemic infection. Spray inoculation produced superficial infection in the early stages, whereas the leaf tissues of plants inoculated by pricking were invaded very rapidly, preventing treatment efficacy, even of the antibiotics ciproflaxacin and tetracycline.

Behlau, *et al.*, (2008) & Graham, and Leite, (2004) reported that the higher incidence of dropped fruits and reduced fruit yield as incidence of foliar infection increased, Copper sprays were demonstrated to reduce canker-induced fruit drop and control of the disease increased as spray interval decreased, and also found that the relationship between disease incidence and fruit yield became weaker as the trees became older. The regular use of copper sprays has a potential to decrease citrus canker incidence in comparison to unsprayed trees (McGuire, 1988). Gottwald and Timmer, (1995) find that Irrespective of the spray frequency, the incidence of leaves with canker on copper-treated trees was never higher than 16%. In contrast, disease incidence was as high as 51% for non-sprayed trees. Roberts, *et al.*, (2008) showed Copper and mancozeb consistently (>90%) reduced the disease severity compared to the untreated control plants. In the tomato bacterial spot field trials, copper-sensitive strains of *Xanthomonas* were inoculated in field plots however, disease suppression was generally more conservative, for example the 32%-76% diseases suppressed.

Gleason, *et al.*, (1993) investigated the products containing copper has reported to significantly reduce foliar leaf, fruit spotting produced by this pathogen.
Copper treatments were more active when mixed with mancozeb, suggesting a synergistic effect because mancozeb alone did not reduce populations or spread. Hausbeck, et al., (2000) reported on \textit{Pseudomonas syringae} pv. \textit{mango} when copper is combined with carbamate fungicides was effective. Ninot, et al., (2002), showed relevant data from using of excess copper applications to crops lead to contamination in soil, it pollutes soil environment and copper tolerance of plant-pathogenic bacteria increased (Andersen, et al., 1991). Consequently, copper applications on commercial crops should be reduced.

Reports drew our attention to develop plant based products for control of bacterial black spot disease caused by \textit{Xcm} in mango plants. In the present study, the activity of the macro fungal and plant-extracts against the bacterial growth of \textit{X. campestris} pv. \textit{mangiferaeindicae}. It was observed that plants parts extracts tested, all plant extracts showed inhibitory effect against the bacterial growth of \textit{Xcm}, the maximum inhibitory effect was shown by fruit extract of \textit{Sapindus laurifolia} and average inhibitory effect was shown by \textit{Xylaria} sp., \textit{Asclepias curassavica}, \textit{Helicteres isora}, \textit{Piper betel}, \textit{Tamarindus indica}, \textit{Tridax procumbens}, \textit{Azadirachta indica}. The test bacterium was less inhibited by leaf extract of \textit{Coffee} leaf, show antibacterial effect against the test bacteria

Medeiros, et al., (2009) showed coffee-leaf extract that has proved to be efficient in the control of several coffee and cotton diseases also exhibits potential for the control of bacterial spot in tomato. Cui, et al., (2010) have shows the susceptibility to the plant extracts of \textit{Clostridium} spp. the combined antibotulinal efficacy of nutmeg, sage and clove extracts observed in the development of
minimally processed meat products, particularly those with low levels of NaN02 (10 ppm)

Ahmad, and Aqil, (2007); Amadioha, (2000) & Samy, and Ignacimuthu, (2000) reported management of plant diseases by application of plant products has suggested (leaf extract of D. metel was found to be more effective in reducing the spread of sheath blight and bacterial blight diseases in rice (Hausbeck, et al., 2000). Kumudini et al., (2001) have reported that the leaf extract of D. metel exhibited 80% protection against the downy mildew pathogen, Sclerospora graminicola, and induced resistance in the highly susceptible HB3 cultivar of pearl millet. Various other plant species have been tested for the control of sheath and bacterial blight diseases. Ansari, (1995), suggested the use of Trachispermum ammi and Ocimum sp. to control sheath blight of rice without any harmful effects to the plant. Similarly, Gangopadhyay (1998), reported the suppression of symptom development in turmeric (Curcuma longa) extract sprayed rice plants when inoculated with Xoo.

Kagale, (2004); Tewari, (1995); & Barbour, (2005) find out several plant species have been screened for antifungal activity and extracts/purified compounds from these plants were found to have a broad spectrum of antimicrobial activity and control pre-harvest and post-harvest diseases of several plant species (Mishra, and Dubey, 1994). Datura metel completely inhibited in vitro conidial germination of C. capsici (Gomathi, and Kannabiran, (2000). Kurucheve, et al., (1997) reported that extracts of L. inermis were inhibitory to R. solani and Bambawale, et al., (1995) reported that ethanol extracts of L. inermis were effective in control of the cotton pathogens Alternaria macrospora, Myrothecium roridum and Xanthomonas compestris in in vitro tests.